



# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

SEPTEMBER, 1947

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# Dosage is Important

Vitamin B<sub>12</sub> is now generally administered in large and sometimes in massive doses. Palmer<sup>1</sup> reported that 40 mg daily by the mouth was effective in a case of anorexia nervosa. American workers in 1939<sup>2</sup> recommended that, in nervous disorders the vitamin be given in doses of 50 mg to 100 mg and in 1941 Sholl<sup>3</sup> suggested an initial amount of 20 mg to 50 mg by injection in definite deficiency.

In severe injury haemorrhage and infection, Levenson and co-workers<sup>4</sup> favour treatment including 50 mg of vitamin B<sub>12</sub> daily during the period of acute stress. After this the doses might be reduced to 10 mg daily. The polyneuritis of pregnancy responds to 5 mg to 20 mg daily and for toxæmia of preg-

nancy 20 mg to 60 mg together with liver extract<sup>5</sup> is suggested.

Schott<sup>6</sup> recommended complete rest with 15 mg to 25 mg vitamin B<sub>12</sub> in certain cases of congestive heart failure.

References—(1) *Lancet* 1939, i, 261. (2) *J. A.M.A.* 1939, 11, 2, 95. (3) *Post Grad. Med.* 1941, 37, 3. (4) *Ann. Surg.* 1941, 124, 840. (5) *Surg. Gynec. Obstet.* 1940, 70, 1433. (6) *B.M.J.* 1941, 2, 1945, 39, 179. (7) *Pharmacol.* 1941, 34, 44.

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TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL 41 No 1 SEPTEMBER, 1947

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LABORATORY MEETING

of the Society held at the  
Royal Army Medical College, Millbank, London,  
on  
Thursday, 20th March, 1947, at 7 30 p m

THE PRESIDENT

C M WENYON, C M G , C B E , M B , B S C , F R S ,  
in the Chair

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DEMONSTRATIONS

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ROYAL ARMY MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

Major J A Manifold

**Anaemia in Indian troops**

Severe anaemia in Indian troops during the war was a major problem as these cases required prolonged hospitalization Charts were presented showing that these men were recruited from a population with an extreme variation in Hb levels, the well-fed Indian's blood level being absolutely comparable with the best European standards

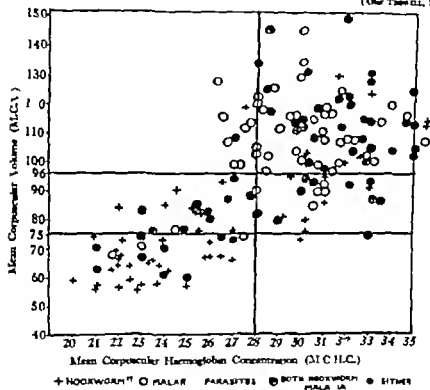
Two main series of cases from base areas in India were compared graphically, one of 166 and the other of 762 None of these men had been exposed to active service conditions In spite of the fact that these troops were in receipt of a diet of some 4,000 calories with 109.7 grammes protein (animal 23.8 grammes), the mean haemoglobin level on reporting sick was 7.22 grammes per 100 ml and the peak incidence was during the 2nd to 3rd year of service



The case incidence was very significantly higher in vegetarians than in non-vegetarians.

Chronic malaria and hookworm infestation were the chief associated aetiological factors. 168 cases were statistically analysed at a mean Hb level of 7.22 grammes, showing a very marked association between chronic malaria and macrocytosis and hookworm with microcytosis and hypochromia. This association did not exist at a high haemoglobin level.

168 CASES OF SEVERE ANAEMIA IN INDIAN TROOPS, CLASSIFIED BY M.C.V. & M.C.H.C.  
(After TAYLOR, 1942)



The suggested aetiology may be expressed in the form of an equation. This is shown in Table I below

TABLE I.

Precipitating factors.	Unknown factor	Substrate	
(1) Chronic malaria and or (2) Hookworm infestation and or (3) Dysentery diarrhoea, etc. (4) Pregnancy	+ ?	Poor intake of protein of high biological value and high phytic acid content of the diet.	→ Anaemia

In view of the widespread nature of the precipitating factors indicated above, which are much more common than are cases of severe anaemia, it is necessary to postulate the action of an unknown factor which appears to be a personal anaemic diathesis, and is possibly conditioned by years of low intake of protein of high biological value. The presence of the unknown factor is needed to explain the fact that if two people were exposed to the same conditions of haematological stress, one might become anaemic while the other might not. Within broad limits, the degree or extent of the precipitating factors do not affect the issue. The mechanism by which each of the precipitating factors might act was shown graphically.

The effect of a diet low in protein was illustrated by showing some details from poor Indian civilian cases, whose mean total plasma protein level was  $5.19 \pm 1.05$  gramme per 100 ml, sixty-six cases in troops were analysed in full haematological detail, which is summarized in Table II.

TABLE II

MEAN HAEMATOLOGICAL FINDINGS IN SIXTY-SIX CASES OF SEVERE ANAEMIA IN INDIAN TROOPS

* Fore treatment ter	R B C m/ $\mu$ l	Hb G /100 ml	M C V	M C H C	M D $\mu$	W B C	Abl eosins	Abl lymphs and monos	Bili- rubin mg %	Plasma pro- tein G %	Days in hos- pital
Group 1—Hookworm and anaemia											
	4.2	6.5	69	23.5	6.7	7,358	1,336	2,358	0.25	6.9	56.4
	5.2	13.3	85	29.1	7.23	7,593	—	—	—	—	—
Group 2—Malaria plus hookworm and anaemia											
	3.0	7.7	97.9	27.4	7.8	8,536	769	2,899	0.7	7.2	68.6
	5.4	14.4	87.0	31.0	7.27	4,900	—	—	—	—	—
Group 3—Malaria and anaemia											
	2.3	7.6	108.3	29.7	7.95	4,813	144	1,972	0.9	6.7	66.0
	4.9	13.5	89.0	31.5	7.43	7,470	—	—	—	—	—
Group 4—Anaemia with neither malaria nor hookworm											
	2.6	7.8	108.4	29.5	8.1	5,331	203	2,079	0.9	6.3	53.3
	4.7	13.3	89.3	31.5	7.18	6,167	—	—	—	—	—

\* The standard deviations are omitted for the sake of clarity

## DEPARTMENT OF HYGIENE

## The new aluminium water bottle

This type of water bottle is gradually replacing the older type. The two types can be compared as under

## (1) Weight.

Old type.		Grammes.	New type.		Grammes.
Bottle		400	Bottle alone		200
Sling	..	100	Bottle with cup		365
			Cover		172
Total		500	Total		537

(2) The wide neck and rounded shape of the new bottle make it more easy to clean, and the screw cap which replaces the cork of the old type is an improvement.

(3) The new bottle also has the advantage of having a drinking vessel incorporated with it.

## Apparatus for sterilizing water and for the manufacture of hypochlorite

A 5 per cent. solution of salt (1 lb. to 2 gallons of water) is drawn by suction from the galvanized iron tank between graphite electrodes in the black carbonite Chlorocell.

The flow of salt solution is adjusted by means of the screw valve on the front of the "Chlorocell" until the rotameter in the tube below registers 100 ml. per minute.

The switch of the variable resistance at the top right corner of the panel varies the electric current as indicated on the ammeter and thus yields hypochlorite in direct proportion to the current.

The salt is electrolysed to sodium hypochlorite a powerful germicide, in its passage between the electrodes.

The hypochlorite is then drawn into the water supply which is rendered sterile after a short period of contact. (The glass vessel holds chlorinated water heavily chlorinated in the exhibit.) This apparatus is designed for a flow of 1 000 to 3,000 gallons an hour.

The apparatus can be modified to make a solution of hypochlorite for hospital use in place of Dakin's or other similar solutions.

## Wide mesh anti-mosquito netting.

One of the greatest drawbacks to the efficiency of insect repellents applied directly to the skin has been the necessity for frequent application in order to maintain the repellent effect.

With the object of obtaining protection for at least one night, experiments have been made with wide mesh netting impregnated with dimethyl-phthalate (D.M.P.) and used as a covering for the exposed parts of the body.

## LABORATORY MEETING

The apertures in the netting are one-quarter of an inch square and the netting provides a marked degree of protection when impregnated with one-half of its weight of D M P. Impregnation can be maintained by storing it in a waterproof container lined with lint soaked in D M P.

In one area with an extremely high biting rate (108 culicines in 15 minutes and 33 anophelines in 20 minutes) such a net gave complete protection for two nights without re-impregnation. Re-impregnation with a smaller dose of D M P (1/10 c c per gramme of net) restored full protection for a further two nights.

Field trials have shown this method of protection to be effective without interfering with hearing, vision, or the handling of weapons.

A modern Sartorius air-damped analytical balance capable of weighing accurately from 100 grammes to 0.1 milligramme and an electrolytic hypochlorite apparatus for sterilizing water, were shown.

## DEPARTMENT OF TROPICAL MEDICINE

Mr John Hull Grundy, Entomologist

A new method of teaching medical entomology

Large working models of Arthropoda of medical importance, made from 1/16-in white cardboard sheets, cut to various shapes, bent to form curved surfaces, coloured with wax-crayons, and with the working parts joined by aluminium rivets, were on view, together with permanent coloured chalk drawings on special blackboards, 8 ft long and 4 ft high, made of blackened Essex-boards nailed to framework. The models and blackboard drawings are normally used during senior-course lectures at the college, in conjunction with demonstrations of actual specimens. Appropriate preparations of these demonstrations were also shown.

## Models of entomological specimens

## ARACHNIDA

- 1 Gorge-mite (*Tyroglyphus*)
- 2 Harvest-mite (*Trombicula*, larva)
- 3 Scabies-mite (*Sarcoptes scabiei* ♂)
- 4 Soft-tick (*Ornithodoros*)
- 5 Hard-tick (*Imblyomma* ♂, ♀, gravid abdomen of ♀)

## INSECTA

- 6 Conc-nose bug (*Panstrongylus*)
- 7 Bed-bug (*Cimex lectularius* ♂, ♀, *Cimex hemipterus* pronotum, egg)
- 8 Body-louse (*Pediculus humanus* ♀, abdomen of ♂, leg, egg)
- 9 Crab-louse (*Phthirus pubis* ♂)
- 10 Flea (*Xenopsylla* ♂, abdomen of ♀, larva, heads of *Pulex*, *Nosopsyllus*, *Pariodontis*)
- 11 Fly (*Schizophora*—head with pulvum and mouth parts)
- 13 *Anopheles gambiae* (external anatomy, lateral view)

## Blackboard drawings

12. Anopheline and culicine larvae compared (dorsal view)

## Specimens

*Trematode akamushi* (in damar).  
*Tyroglyphus longior* (in P.V.A. mountant)  
*Sarcoptes scabiei* (in damar)  
*Amblyomma* (dorsal and ventral view of capitulum, in air)  
*Pediculus humanus* (life history in 3 per cent. formalin)

Anopheles and culicine larvae (living in partitioned water-container)  
*Culex pipiens* (lateral view in 75 per cent. alcohol fluid-mount)  
*Calliphora* (frontal view of ♂ and ♀ heads, in air).

## Dr A R D Adams and Dr D R. Seaton

The results of intensive treatment of six cases of kala-azar with sodium antimonyl tartrate

Case histories and temperature charts were shown from six cases of Indian kala-azar treated with a 2-day course of sodium antimonyl tartrate on the lines described by ALVES and BLAIR (1946)\* for schistosomiasis.

The total dose of the drug was approximately 12 mg per kg of body weight, given in six intravenous injections at 3-hourly intervals on 2 successive days.

Four of the six cases were apparently cured by a single course. One case relapsed after a clinical improvement lasting 6 weeks, but was cured by a second course. The remaining case showed no improvement whatever after the antimony and was subsequently treated with pentamidine.

Toxic symptoms were not severe. All cases developed paroxysms of coughing immediately after one or more of the injections. In several instances this was followed by vomiting. One case developed a toxic jaundice 2 days after the injections. It cleared up in the course of a fortnight.

## Dr D S Bertram

The infectivity of *Liponyssus bacoti* with *Leishmanoides curvica*.

Since the original observations by WILLIAMS and BROWN (1945)† on the role of the tropical rat mite, *L. bacoti* as a vector of the filarial worm, *L. curvica* to cotton rats, numerous workers in this country and in America have studied the infection with a view to its use in chemotherapeutic research. Methods of feeding known numbers of mites on white rats and cotton rats at known intervals were developed by BERTRAM, UNKSWORTH and GORDON (1946)‡

Tables were given of a result obtained using these methods in which mites infected from a cotton rat with a high microfilaria count (2,625 mf./1 c.mm.) showed a high percentage rate of infection (33 per cent.). The mites were infective to rats as early as the 15th day (or third meal after becoming infected) and retained their infectivity until at least the 25th day (fifth meal).

ALVES, W & BLAIR, D M. (1946.) The intensive treatment of schistosomiasis with antimony. *Lancet*, 1 1 p. 9

† WILLIAMS, R. W & BROWN H W. (1945) *Science* 101, 482.

‡ BERTRAM D S, UNKSWORTH, K. & GORDON, R. M. (1946) *Ann. trop. Med. Parasit.*, 40 223.

In cotton rats, microfilariae were found 53 and 54 days after exposure to potential infection by seventeen to thirty of the mites. There was some indication that maximum transmission occurred on the 15th day but that transmission was appreciable also on the 20th day.

The results suggested that to obtain a maximum infection in a cotton rat, infected mites should be fed on a single uninfected host on at least both the 15th and 20th days after the mites' infecting meal.

Prof J J C Buckley

1 *Coenurus* from human spinal cord (Specimen presented by Mr D W C NORTHFIELD, M S, F R C S, Neuro-surgical Department, The London Hospital)

The exhibit comprised portions of a *Coenurus* cyst which was removed by operation from the spinal cord of a girl of 14 years who had never been outside Britain. The infection may have been incurred during a period of evacuation in 1943-45 to Wales, where there is a history of contact with a dog. Symptoms of paraplegia appeared in December, 1946. Two previous cases are on record of *Coenurus* in human brain, this is the first of its kind from the spinal cord.

2 *Cysticercus bovis* in the liver of a giraffe

The material was obtained from the liver of a giraffe which died recently at the Zoological Gardens, London.

3 *Trichostrongylus asymmetricus* from the stomach of a wallaby

This interesting species was first found and described by T W M CAMERON in 1926, from the stomach of a wallaby which died in the Zoological Gardens, London. It was recently found in the same host at the Zoo, but in addition to the adult worms lying in the mucosa of the stomach, immature forms were found encysted in the non-gastric mucosa. This is an unusual phase in the development of a species of *Trichostrongylus*.

Dr F Hawking, Mr P Sewell and Miss P D Davey

The maintenance of a filarial infection (*Litomosoides carini*) in the laboratory (*L. carini*) is a filarial infection which occurs in the cotton rat (*Sigmodon hispidus*). It is transmitted by the tropical rat mite, *Liponyssus bacoti*, which lives in the nests of wild cotton rats.

Arrangements have been made at the National Institute for Medical Research to maintain this infection and to transmit it to cotton rats on a large scale, so as to provide a supply of infected rats for investigations on the chemotherapy of filariasis.

Briefly, the procedure is as follows. Mites in the bedding are infected by allowing them to feed for 1 to 2 weeks on rats containing microfilariae in the blood. Clean rats are then placed for 2 weeks on the bedding containing these infected mites so that transmission can occur. The rats are then de-mated and stored for 2 to 2½ months until the worms mature in them and microfilariae are found in the peripheral blood stream. The rats are then ready for experimentation.

Photographs were shown demonstrating these arrangements and an adult worm, maintained alive *in vitro* in a medium of serum and Tyrode solution was exhibited.

Dr P. L. Le Roux

- 1 Section of skin and subcutis of an ox showing *Glabidum* cysts and microfilariae of *Oncocerca guthriei* Neumann 1910 Mashai River area, Livingstone Northern Rhodesia.
- 2 Portion of subcutaneous muscle showing infestation of the connective tissue with the cysts of (?) *Glabidum bonatti* Marechal 1912.

These cysts have been observed in the connective tissue throughout the body and in the nasal mucosa. The parasite is of common occurrence in cattle from Bechuanaland Protectorate Caprivi Strip in South-West Africa and parts of N Rhodesia. It has been recorded from the Transvaal and probably has a very wide distribution in Central Africa.

3. Portion of body of a female *Oncocerca* *sp.* from a subcutaneous nodule in a man from Gambia, showing two "secondary striations" to each inter-rustral space.

These subcuticular striations are analogous to the transverse striations of the Trichostrongylidae and some other nematodes. Two subcuticular striations to each inter-rustral space have hitherto only been recorded in *O. caecitarsis* of man from Central America and in *O. gibsoni* of cattle, both of which occur in nodules and in *O. leuvalis* which occurs unencapsulated in the gastro-splenic ligament and in the capsule of the spleen of cattle in the United States of America, and in Australia and England.

- 4 Portion of a female of an *Oncocerca* *sp.* from a subcutaneous nodule in the region of the brisket in a small African antelope a duiker (*Cephalophus griseus*) at Mtek Isaka district, N Rhodesia.

Specimens have been collected from the same host and other duikers in various parts of N Rhodesia and once in Zululand, Natal. This species from nodules in duikers is morphologically closely related to the nodule-forming species on man and cattle but occurs in areas where cases of onchocerciasis in man have not been recorded.

5. Intramuscular onchocerciasis in an ox, Northern Rhodesia.

This form of onchocerciasis has at times been mistaken for *Cysticercus* *locus* infestation. All muscles of an animal may be infested. Cases have been observed amongst slaughter animals imported from Bechuanaland Protectorate and Southern Rhodesia. It also occurs in cattle in parts of Natal, Portuguese East Africa and West Africa (Gold Coast Colony). The parasite is generally accepted to be *O. gibsoni*. In Southern Africa no cases of human onchocerciasis have been observed in the endemic areas.

- 6 Portion of a female of *Onchocerca cervicalis* without cuticular thickenings from the connective tissue next to the funicular portion of the ligamentum nuchae of a mule, Mazabuka, Northern Rhodesia

In the anterior and the posterior portions of the worm the cuticular ridges were present. The same phenomena were observed in specimens collected from four horses in London. The parasites were present in the connective tissue between the elastic fibres of the ligament and in the connective tissue next to the ligament. Most of the parasites were encountered in the last-mentioned habitat which should probably be accepted as the normal location of this species in the horse.

- 7 Proximal part of the M biceps brachii from an ox showing *Onchocerca gutturosa* infestation, Mazabuka, Northern Rhodesia

This species seems to have a worldwide distribution and if beef, infested with it, is to be condemned as unfit for human consumption the breeding of cattle for beef production will become uneconomical until the breeding of vector has been eradicated. In Northern Rhodesia the vector would seem to be a species of *Culicoides* and not *Simulium* sp. which is regarded as the intermediary in England. It seems possible that the larval stages of the *Onchocerca* sp. encountered in a *Simulium* sp. after having been fed on an infected cow may have been those of *O. lienalis* which is by some workers regarded to be synonymous with *O. gutturosa*. These two species are morphologically distinct.

- 8 *Onchocerca* sp. in the tendon of insertion of the M rectus femoris of a red lechwe (*Onotragus lechwe*), a species of kob, Kafue River, Mazabuka, Northern Rhodesia

This parasite occurs in several other species of antelope in Northern Rhodesia. Morphologically, it is more closely related to *O. reticulata*, *O. cervicalis*, *O. gutturosa*, *O. flexuosa* and *O. fasciata* than to *O. volvulus*, *O. lienalis*, *O. gibsoni* and the species from the nodules in the duiker.

- 9 Specimens of an *Onchocerca* sp. on the lateral surface of the M biceps femoris close to the stifle (knee in man) joint in a roan antelope (*Hippotragus equinus*), Chunga Ranch, Chinsali district, Northern Rhodesia

This species would seem to be identical with the species parasitizing the red lechwe and the hartebeest.

- 10 Portion of skin, fixed in formalin and dried, showing calcified and uncalcified specimens of *Onchocerca* sp. in the subcutis in the region of the pastern of the front limb of a Lichtenstein's hartebeest (*Alcelaphus lichtensteini*), Chunga Ranch, Chinsali District, Northern Rhodesia

Dr J Makari

Serial cephalin flocculation curves, application in the study of tropical diseases and their relation to a new resistance factor



In this work, the serial dilution method (MAKARI 1946) was followed using the tubes instead of Hanger's original one-tube method. The serial cephalin flocculation (S.C.F.) curves were obtained by plotting the degree of flocculation as ordinate against the serial dilutions as abscissa.

Four main S.C.F. curves were noted —

- (1) S.C.F. Curve I.—Found in healthy volunteers.
- (2) S.C.F. Curve II.—Found in patients with liver disease
- (3) S.C.F. Curve III.—Found in cases with reticulo-endothelial stimulation.
- (4) S.C.F. Curve IV.—Found in allergic and some endocrine diseases studied.

The mechanism underlying the S.C.F. curves was demonstrated, as well as the experimental production of these curves. Evidence was presented that the inhibiting factor revealed by the S.C.F. curves which was shown by DENNIS and MAKARI (in press) to be in the albumin residue and which is responsible for inhibiting the flocculating activity of the  $\gamma$  globulin, is in fact a *resistance factor* of importance in determining the course and outcome of this struggle between host and parasite. The different clinical manifestations in malaria, the therapeutic effect of quinine and atehrin as well as the clinical outcome of infection in man, may be explained on the efficiency or inefficiency of this *resistance factor*. Fundamental differences were observed between *P. vivax* and *P. falciparum* infections. In the latter a much more marked initial depletion and a much less satisfactory response of this *resistance factor* was noted when the antimalarial drugs were used. An immediate and transient increase in this resistance factor was brought about by vaccination with T.A.B., and a much less resistance factor depletion was noted when vaccinated individuals succumbed to the typhoid infection, as compared with unvaccinated individuals.

A more detailed account is in the press (*Nature*).

Dr P. E. O. Manson-Bahr

A series of temperature charts of cases of epidemic thrombophlebitis in East African soldiers following vespanoture.

Five charts were shown representing the three main types of this syndrome. Thrombophlebitis, relapsing pyrexia with stiff neck, relapsing pyrexia without phlebitis or stiff neck, relapsing.

Dr Kenneth Mellanby

Effects of mosquito bites on man.

Different individuals are known to give very varied reactions to the bites of blood-sucking insects. Experiments have shown that with several species

MAKARI, J. (1946) *J. trop. Med. Hyg.* 49 113

## LABORATORY MEETING

of mosquito a series of different reactions is given depending mainly on the extent to which the individual has been exposed to the bites of that species. There is a distinct immediate reaction which takes the form of a raised welt. This subsides and may be replaced by an itching welt 24 hours later. Four distinct stages are found in most individuals and may be summarized as follows:

Stage	Immediate reaction	Delayed reaction	Stage	Immediate reaction	Delayed reaction
I	—	—	III	—	—
II	—	—	IV	—	—

Volunteers had been exposed 24 hours before the meeting, and immediately preceding the demonstration to bites from *Aedes aegypti*, *Anopheles maculipennis* and *A. quadrimaculatus* and all stages of reaction were shown. It was noted, for instance, that the same individual gave Stage I to *Aedes aegypti*, Stage III to *Anopheles quadrimaculatus*, and Stage IV to *A. maculipennis*.

Dr G. G. Mer (shown by Dr C. M. Wenyon).

Exoerythrocytic schizogony in bat malaria. Films of the heart blood and of the internal organs (liver, kidney, lung, bone marrow) of *Myotis myotis* of Palestine were shown. In the blood films only male and female gametocytes were present, there being no evidence of erythrocytic schizogony. In the organ smears, however, non-pigmented exoerythrocytic schizonts in various stages of development were clearly seen. These occur in macrophages and other cells. It is evident that the schizogonic cycle of this parasite does not occur. A preliminary note on these findings was published in *Nature* (1947), 159 (March 29), 441.

Air Commodore T. C. Morton

1. A case of amoebiasis of the caecum. This man, aged 41 years, was admitted to a R.A.F. Station Hospital on 11.1.47. Since Christmas, 1946, he had complained of vague abdominal pain, flatulence and loss of appetite. The day before admission the pain had become more severe, there was no history of bowel disturbances. On direct questioning, he stated that he had not been abroad. (There is reason to believe that he may have served overseas in the Army prior to R.A.F. enlistment but so far this has not been confirmed.) Owing to the presence of pus cells and staphylococci in his urine, he was treated for a urinary infection with penicillin. On 16th January his condition rapidly deteriorated and there was marked tenderness over the right iliac fossa where an ill-defined fullness was evident. A laparotomy was carried out and an inflammatory mass was found involving the caecum and ascending colon, which showed two areas of necrosis through which faecal contents escaped. A right hemi-colectomy was performed with

an end to side anastomosis to establish bowel continuity. Death occurred on 20.1.47.

Examination of the caecum revealed a large necrotic area which had perforated on the posterior aspect near the ileo-caecic junction. The whole caecum was greatly thickened, in some areas being over 1½ inches in thickness whilst the entire mucosa was replaced by extensive ulcers with thin islets of epithelium intervening. Sections revealed numerous *Entamoeba histolytica*. The appendix was slit up but no macroscopic evidence of ulceration of the mucosa was visible and sections failed to reveal any *E. histolytica*.

#### Postmortem

A well shut off subphrenic abscess on the right side was present. Leakage had also occurred from two ulcers just below the splenic flexure where a pericolic abscess had formed. The liver was normal. The large bowel was studded with numerous ulcers of varying sizes from the walls of which *E. histolytica* were demonstrated.

The interesting features in this case are that the deceased was at full duty up to the day he reported sick on 11.1.47 in spite of the extensive involvement of his colon, and his categorical denial that he had ever served overseas, although, as already stated, there is an element of doubt as to the veracity of this statement.

#### 1. A case of amoebiasis of the buttocks.

This airman, aged 27 years, noticed a lump in his left buttock 3 weeks after his arrival in England after a tour of 19 months in India. Apart from some pain on sitting this gave him no trouble and did not interfere with his normal activities. Four months later the swelling burst and discharged pus, after which the pain became more severe, and he was admitted to hospital on 4.1.47. The abscess was incised on 8.1.47 and two main cavities were found communicating with each other. Pus from the sinus grew *Staphylococcus aureus*. Healing was retarded, and in spite of a full course of penicillin the abscess had to be re-incised on 21.2.47. Curettings from the numerous sinuses were sent to the R.A.F. Institute of Pathology and Tropical Medicine and *E. histolytica* were seen in the sections. The patient was transferred to the Princess Mary's R.A.F. Hospital, Halton and active *E. histolytica* were found in the curettings from the sinuses. Sigmoidoscopy showed two small petechial haemorrhages on the anterior wall of the rectum but no communication with the abscess could be detected. Scanty *E. histolytica* cysts of a minute strain were present in the faeces though the patient stated he had never suffered in the past from dysentery or severe diarrhoea.

#### Mr P. G. Shute

Specimens of blood parasites stained with Leishman stain which had retained their colour for many years.

- ears ) Sporozoites  
8 Trypanosoma sp. Stained and mounted  
9 P. cynomolgi
- Stain used** Leishman (Baird and Tatlock)  
The Leishman powder was added to the methyl alcohol without grinding in a mortar and the stain was not filtered. Such stain is ready for use after 24 hours and retains its full staining properties for at least 6 months in our climate. The distilled water used for diluting had a pH of 7.2

**Technique** Without previous fixation, four drops of stain were dropped on to the smear and allowed to act for from 15 to 20 seconds—not longer. Twelve drops of distilled water were added, a dilution of three to one was found to give the best results. The mixture was allowed to act for from 15 to 30 minutes, 15 minutes for *P. vivax*, *P. ovale* and trypanosomes, and 30 minutes for *P. malariae* and *P. falciparum* parasites. After staining, the films were washed in a stream of distilled water for 5 seconds and then dried. When quite dry they were mounted in green euparal (euparal vert).

While distilled water which has been buffered to neutral or very slightly alkaline pH was satisfactory results, such films do not keep well for long periods. The distilled water used for diluting had a pH of 7.2 (using lithium carbonate) and the distilled water to which a pH of 7.2 was brought by the addition of a few drops of sodium hydroxide solution kept for several months on coal fires without any noticeable change in pH.

Without adding anything from outside, a dilution of three to one (v/v), 15 minutes after the parasites are added, a dilution of three to one (v/v) is made. The slides are allowed to act for from 15 to 30 minutes, and *P. falciparum* parasites are removed, and 30 minutes for *P. malariae* and *P. falciparum* parasites are removed, and they were washed in a stream of distilled water for 5 seconds and then dried. While distilled water which has been buffered to neutral or very slightly alkaline (pH 7 to 7.2) gives satisfactory results, such films do not keep well for long periods. The best results have been obtained with distilled water which has a pH of 7.2 (using lithium carbonate). It is not, however, sufficient to bring the distilled water to a pH of 7.2 and immediately carry on with the staining because under certain conditions the distilled water quickly becomes acid, e.g., where there are Bunsens, gas fires or even coal fires. When films are being stained specially for keeping purposes, the distilled water is brought to a pH of 7.2 and a few c.c. are poured into a petri-dish and placed on the bench exposed to the atmosphere for the same length of time as it is proposed to stain the films. At the end of the time the pH of the water is again tested and if it has become acid more alkaline is added until such time that it is neutral or very slightly alkaline after 15 to 30 minutes, according to the proposed staining time.

It was found that in the summer months distilled water with a pH of 7.2 often became acid within a very short time when exposed to the atmosphere for an hour or more, whereas in the winter months it remained at a pH of 7.2 for several days.

The result of the oil immersion lens examination of the films stained with the oil immersion lens was as follows:

Unmounted films which have been examined with the oil immersion lens may, or may not, keep well after subsequent mounting in euparal, the result depending, apparently, on whether the oil or xylol used is acid. It therefore

seems advisable to mount with euparal, before examining with an oil immers on lens, films which are to be preserved for a long time. Xylol tends to become acid if kept in partially filled bottles (LEE's *Lade Medien* p. 72). This being so films although neutral, may be rendered acid when xylol is used to dissolve the oil and fading will quickly occur even though a neutral mounting medium is used.

If films are bright pink after being stained, this is proof that the staining solution is acid and such films will soon fade, beginning with the cytoplasm. The general appearance of films which keep well is a pale slate colour.

Mr J S Steward

The mounting of biological specimens in perspex (methyl methacrylate monomer)

### *Preparation of the Monomer*

Methyl methacrylate monomer is stabilized by the addition of hydro-quinone which has to be removed before thickening and setting can occur.

Removal of the hydro-quinone may be effected by distillation or washing twice with 5 per cent. caustic soda in separating funnel—running off the heavier caustic. The monomer is then washed until the liquid is no longer alkaline (test with phenolphthalein).

The methyl methacrylate monomer is then run off and dehydrated with anhyd calcium chloride for 24 hours. It is then filtered through filter paper and used, or stored in a refrigerator.

### *Preparation of Embedding Syrup*

A mixture containing 85 parts by volume of washed methyl methacrylate monomer containing 0.02 per cent. benzoyl peroxide (by weight) and 15 parts dibutyl-phthalate, is placed in flask (which should only be half filled) fitted with an air condenser. Using boiling water bath thickening takes about 30 minutes or with an oil bath at 130° C. about 15 minutes. The mixture should be shaken three or four times during the process. If the reaction becomes violent the flask should be immersed in cold water which should be kept at hand for the purpose.

The monomer is thickened to the maximum convenient viscosity but it should be remembered that the syrup thickens further in cooling. It should be stored in refrigerator until used.

### *Preparation of Specimens*

Specimens fixed by one of the usual methods must be completely dehydrated as any residual moisture causes clouding of the perspex. Before embedding the specimen may be soaked in unthickened monomer or in 10 per cent. solution of benzoyl peroxide in chloroform or boiled for a few seconds in benzene to remove air and aid penetration of the monomer. After boiling in benzene the specimen is placed for a few moments in two successive baths of monomer.

Air may also be removed by subjection of the specimen after embedding in the partially polymerized monomer to a pressure of 30 cm. Hg. in a vacuum deaerator for a period depending on the size of a specimen.

### *Mounting the Specimen.*

Embedding cells may be made from sheets of glass held together by

## LABORATORY MEETING

cellulose tape. Hollow specimens and soft tissues are impregnated with monomer of slightly less viscosity than that used in embedding. The specimens are, if small, affixed with perspex cement to a piece of sheet perspex, or, if large, tied down with silk. The sheet perspex may be cut to a suitable size with a fine saw. The specimen is placed in the cell which is then filled with the thickened monomer, and is left at room temperature until all visible air bubbles have risen to the surface, before covering the surface with a weighted sheet of glass.

A considerable depth of monomer should be left above the specimen to allow for the contraction taking place during polymerization.

The use of a vacuum pump has been suggested in drying the specimens under vacuum before impregnating with monomer, and immediately after embedding it in the syrup to reduce bubble formation to a minimum.

*Polymerization*

The specimens may be kept at 40° C until polymerization is complete or after partial polymerization the temperature may be raised to 50° C to accelerate the final hardening. The time for polymerization varies from 1 to 3 weeks.

*Finishing*

The polymerized block is cut with a fine band saw as necessary to obtain a symmetrical block. The surface is polished with the finest possible emery papers and finished with perspex polish (or, in default, Bluebell metal polish).

## Mr F L Vanderplank

The chromosomes and part of the sexual cycle of *Trypanosoma rhodesiense*

Ten preparations were shown in this demonstration. Briefly, they were —  
(1) Living *Trypanosoma rhodesiense* under 1/12 and green filter, where under optimal conditions it is possible to see the chromosomes in the living nucleus

(2) Living *T. rhodesiense* under the phase-contrast microscope loaned by Messrs Cooke, Troughton & Simms, Ltd, and set up by Mr E W TAYLOR. This technique reveals all the various structures that can be seen in stained preparations and others which staining does not reveal.

(3) *T. rhodesiense* stained by usual methods and showing the three types of individuals. Adults or long forms, intermediate or female gamont, short, stumpy or male gamont.

(4) *T. rhodesiense* stained by the feulgen technique and light green counter-stain. This stains the nuclei in prophase, anaphase and telophase.

(5) *T. rhodesiense* stained by aceto-carmine, which gives a similar picture

to the feulgen technique but no suitable counter stain has yet been found, so only nuclear material stains.

(6) Showed the chromosomes of the dividing adult, after wet fixation and hydrolysis in normal hydrochloric acid at 60° C. and stained with Giemsa.

(7) The chromosomes of the mature female gamont or gamete showing the three chromosomes in prophase

(8) Preparations showing the nucleolus after wet fixation in Smith's 1940 formula (see DARLINGTON and LA COUR (1947) *The Handling of Chromosomes* London), and hydrolysed in normal hydrochloric acid at 60° C. for 5 minutes, mordant 1 per cent. ammonium molybdate 2 minutes and stained with Giemsa. The nucleolus appears as a bright red body in the interphase or resting nucleus

(9) Showing a mitotic division, anaphase, of a male gamont (short form).

(10) Showing the latent bodies formed after sexual union of male gamete and female gamete with one or two nuclei and six chromosomes in various stages

The purpose of the demonstration was to show some of the methods used and the type of preparation obtained in studying the chromosomes and sexual cycle of *T. rhodensis*. A full record of data obtained on this subject is at present in preparation for publication.

## ORDINARY MEETING

of the Society held at  
Manson House, 26, Portland Place,  
on

Thursday, 10th April, 1947, at 8 p m

THE PRESIDENT,  
C M WENYON, C M G , C B E , M B , B S C , F R S ,  
in the Chair

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THE PRESIDENT We are very fortunate this evening in having Professor CULBERTSON with us. We have heard of the most interesting work that he and his colleagues have done on *Litomosoides carini* in cotton rats, and subsequently of filarial disease in human beings. He has produced the first definite results in the treatment of filariasis, and, as he says in his paper that the treatments are not final and that it is hoped that better treatments are to come, we hope he will be able to continue his valuable work. We shall be very glad to hear from him now some account of the experiments he has so successfully carried out.



## PAPER

## EXPERIMENTAL CHEMOTHERAPY OF FILARIASIS

BY

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in collaboration with*

HARRY M. ROWE,

*Department of Medicine, Columbia University, New York*

FEDERICO HERNANDEZ-MORALES and JOSE OLIVERA-GONZALEZ

*Departments of Medicine and of Medical Zoology,  
School of Tropical Medicine, San Juan, Puerto Rico  
and*LUIS FIGUEROA ORTEZ, FRANCISCO RUIZ REYES and ROBERTO NETTEL,  
*Mexican Government Hospital for Onchocerciasis Investigations,  
Huatla, Chiapas, Mexico*

Before reading my paper this evening, I wish to express to the Council and to the Fellows of the Royal Society of Tropical Medicine and Hygiene my gratitude for the privilege of being here. I know my several co-workers who are named in the respective sections of the work with which each had to do will take pride in the fact that you have seen fit to ask that the paper be read.

Perhaps no subject in tropical medicine is, by tradition, more appropriate to this Society of which Sir PATRICK MANSON was the first President, and to Manson House which was erected in his memory than is filariasis and surely no aspect of filarial disease would have appealed more to MANSON than a means of eradicating it from infected subjects. It is my purpose this evening to present for your consideration data which have been collected in the course of a fairly large project on the experimental chemotherapy of certain varieties of filarial infection. I believe the data represent a true, precise and logical record of our observations. I think I may safely state that these data indicate that, under the experimental circumstances which are described, it is often possible adversely to affect filarial parasites and occasionally possible to eliminate at least one variety of these worms from subjects by the intensive application of specific drugs. However I wish particularly to make clear that at this time the routine use of the methods here described is not recommended.

This study was supported by grants to Columbia University from Winthrop Chemical Company Inc., and Winthrop Products, Inc., of New York City.

<sup>†</sup> Fellow 1947 of the John Simon Guggenheim Memorial Foundation, New York City.

to the general practitioner in the tropics or elsewhere for the treatment of any of the varieties of filarial disease. I trust that you will assess the information I am about to offer, therefore, with the full understanding that it is derived from a strictly experimental study. What practical application, if any, the procedures described may ultimately deserve remains to be determined.

I propose to discuss the several parts of our work in the following sequence:

- I Chemotherapy of filariasis (*Litomosoides carini*) in cotton rats,
- II Chemotherapy of *Wuchereria bancrofti* infections in man,
- III Chemotherapy of *Loa loa* infections in man, and
- IV Chemotherapy of *Onchocerca volvulus* infections in man

## I CHEMOTHERAPY OF FILARIASIS (*LITOMOSOIDES CARINII*) IN COTTON RATS (*SIGMODON HISPIDUS*)

All of the work I am about to offer stems from the observation made originally in my laboratory in 1943 that the intensive administration of pentavalent antimony eliminates from certain small rodents known as cotton rats (*Sigmodon hispidus*) a filarial parasite (*Litomosoides carini*) which a high percentage of these rodents naturally harbour when caught in the field (CULBERTSON and ROSE, 1944, CULBERTSON and PEARCE, 1946). In this filarial infection, the adult phase of the worm resides free in and usually dispersed throughout the pleural space of the cotton rat and microfilariae appear constantly, without significant periodicity, in the peripheral blood. After intensive treatment with such antimony compounds as neostam, neostibosan, and stibanose (solustibosan), the adult parasites are soon (i.e., in 2 weeks or less) killed, this effect sometimes being attained following a single massive dose of drug. The microfilariae, however, are much more difficult to affect permanently, and although their number in the peripheral blood may drop during the short period of drug treatment, it often rises again to near the pre-treatment level as soon as administration of drug has ceased. Gradually thereafter, however, in animals whose adult filariae have been killed by the drug, the number of microfilariae in the blood declines until none can be found. Sometimes several months are required to elapse before the blood of the treated animal is clear. Once the blood is negative for embryos, however, microfilariae do not reappear in it. If such an animal—that is, one whose blood has finally become negative for microfilariae—be autopsied and its pleural space searched for adult filariae, these will, if found, almost invariably show evidence of having been long dead—considerable disintegration and resorption of the worms having taken place. If a well-treated animal be autopsied shortly after intensive therapy has ceased and while microfilariae are still numerous in the peripheral blood, one can expect to find the adult worms dead in the pleural space and collected into masses covered with fibrinous exudate, these masses of dead worms evidently eliciting a typical foreign body response from the host tissues.

From the foregoing and from the detailed data given in the papers previously cited, it is evident that the adult parasites in the cotton rat filariasis are substantially more vulnerable to the effects of therapy with pentavalent antimony as described, than are the microfilariae. The disappearance of microfilariae from the circulating blood of treated animals, in fact, seems largely to be not the result of a direct action of the drug upon them, but rather the indirect effect of the destruction of the adult worms by the drug. After the death of the mature parasites, microfilariae will no longer be produced. Hence, the blood will be negative for filarial embryos when those present in the circulation at the time the parent worms die are destroyed by whatever antagonistic forces the host animal is able to bring against them.

Following this preliminary work on treatment of the cotton rat filariasis, which has just been described, it was decided to investigate the possibilities of treating human filarial disease. During the last 3 years (since March, 1944) such work has been carried on, and I wish tonight to offer data on patients who have undergone treatment for the presence of any of three species of filarial worms *Wuchereria bancrofti*, *Loa loa*, and *Onchocerca volvulus*. Decidedly the largest part of the effort has gone into treatment of the bancroftian disease, and an extended description of this work will first be offered.

## II. CHEMOTHERAPY OF *WUCHERERIA BANCROFTI* INFECTIONS IN MAN

(In collaboration with HARRY M. ROSE, FEDERICO HERNANDEZ MORALES,  
and JOSE OLIVER GONZALEZ)

The work on treatment of patients with *Wuchereria bancrofti* infection was carried on in Puerto Rico, West Indies, upon native subjects in the University Hospital at the School of Tropical Medicine San Juan, or at certain Insular Homes for Children near San Juan. Since other investigators had, meanwhile, reported the successful therapy of cotton rat filariasis by the administration of different antimony compounds from those tried in my laboratory as well as by arsenical preparations (*Reports to the Office of Scientific Research and Development* 1944 and 1945), it was decided to use a fairly large series of these drugs for treating the human disease. Altogether the work in human subjects, therefore involved use of seven antimony-containing drugs and one arsenical.

All of the patients with *W. bancrofti* have been observed for at least 1 year and some have been under observation for 2 full years since treatment ceased. Four progress reports have already been written from the work, these offering data on certain patients 6, 12 months (CULBERTSON, ROSE and OLIVER-GONZALEZ, 1945), 15 (CULBERTSON, ROSE and OLIVER-GONZALEZ, 1946), and 18 (CULBERTSON, ROSE, HERNANDEZ MORALES, OLIVER-GONZALEZ and PRATT 1946) months respectively after treatment ended.

## MATERIALS AND METHODS

*The Patients*—All of the 129 patients were native Puerto Ricans (excepting one, who was from Martinique) whose nocturnal blood harboured microfilariae of *W. bancrofti*. All were in comparatively good health and only three (two with recurrent lymphangitis and swelling of the legs, one with chyluria) showed symptoms of filarial infection. Many of the patients (those below 18 years of age) were students in children's homes near San Juan, most of the remainder (18 to 38 years old) were males who had generally first learned of their infection when examined by insular military authorities for possible service in World War II. One group of fifteen patients was kept untreated as a control on the infection and on the effects of treatment.

*The Drugs Used*—Four pentavalent antimony compounds (neostibosan, neostam, urea stibamine, and stibanose), three trivalent antimony preparations (fuadin, anthiomaline, and tartar emetic), and an arsenical (melarsen oxide) were employed in this study. All of the drugs were injected intravenously except stibanose, fuadin, and anthiomaline, which were given intramuscularly. Melarsen oxide was administered orally to a few patients and intravenously to others.

*Estimation of Level of Infection*—The level of the filarial infection in patients was determined by counting all microfilariae in 60 c mm. of nocturnal blood, the blood being drawn from a given patient at precisely the same hour for all observations. The dried blood films (20 c mm of blood let dry on each of three microscope slides) were dehaemoglobinized in distilled water, fixed in equal parts of ether and absolute alcohol, stained with Bullard's haematoxylin, and destained as necessary in dilute acid-alcohol. The films were searched for microfilariae under low power ( $\times 100$ ) of the microscope.

From most of the patients whose 60 c mm blood samples were negative, 10 c c of nocturnal blood were also examined, these bloods being obtained at 10 p m or soon thereafter. These large blood samples were first treated with 2 per cent saponin in physiological salt solution. The sediment which remained after saponin treatment was washed with salt solution and centrifuged several times, then examined for the presence of microfilariae under the low power ( $\times 100$ ) of the microscope.

## RESULTS OBTAINED WITH SPECIFIC DRUGS

The first patients studied were treated with neostibosan, this drug being given in the *per diem* dose usually recommended for kala-azar, although for a considerably longer time than required for that infection. The patients, with a single exception, were not hospitalized during treatment, but continued throughout the course of therapy their usual activities as workmen or as students. Subsequently, other patients were placed under much more intensive therapy either with neostibosan or one of the other drugs, the intention being in the later work to apply each drug to near the limit of the patients' tolerance for a

period of usually 2 weeks. Most of these patients were hospitalized during the entire course of treatment. During intensive therapy two doses of drug were generally given daily injections being made at 8 a.m. and 4 p.m. In the few cases where three daily injections were made the third was given at 11 a.m. The precise doses administered on each day of treatment, as well as the reactions to drug seen during therapy have been presented in detail for each patient in an earlier publication (COLBERTSON *et al.* 1946).

#### NEOSTIBOSAN (Winthrop Chemical Company).

Altogether three groups of patients totalling thirty five individuals were treated with neostibosan. Group I, which consisted of twenty persons, were given a comparatively light course of treatment, most patients receiving from 6 to 9 grammes of drug in from 33 to 58 days. The ten persons of Group II were given the same light course of treatment, but 9 months later were intensively retreated (9 to 12 grammes of drug) for a period of 2 weeks. Group III, consisting of five persons, were given a single intensive course of therapy during 2 weeks. The essential information on the patients, the administration of drug, and the results obtained are summarized in Tables I, II and III.

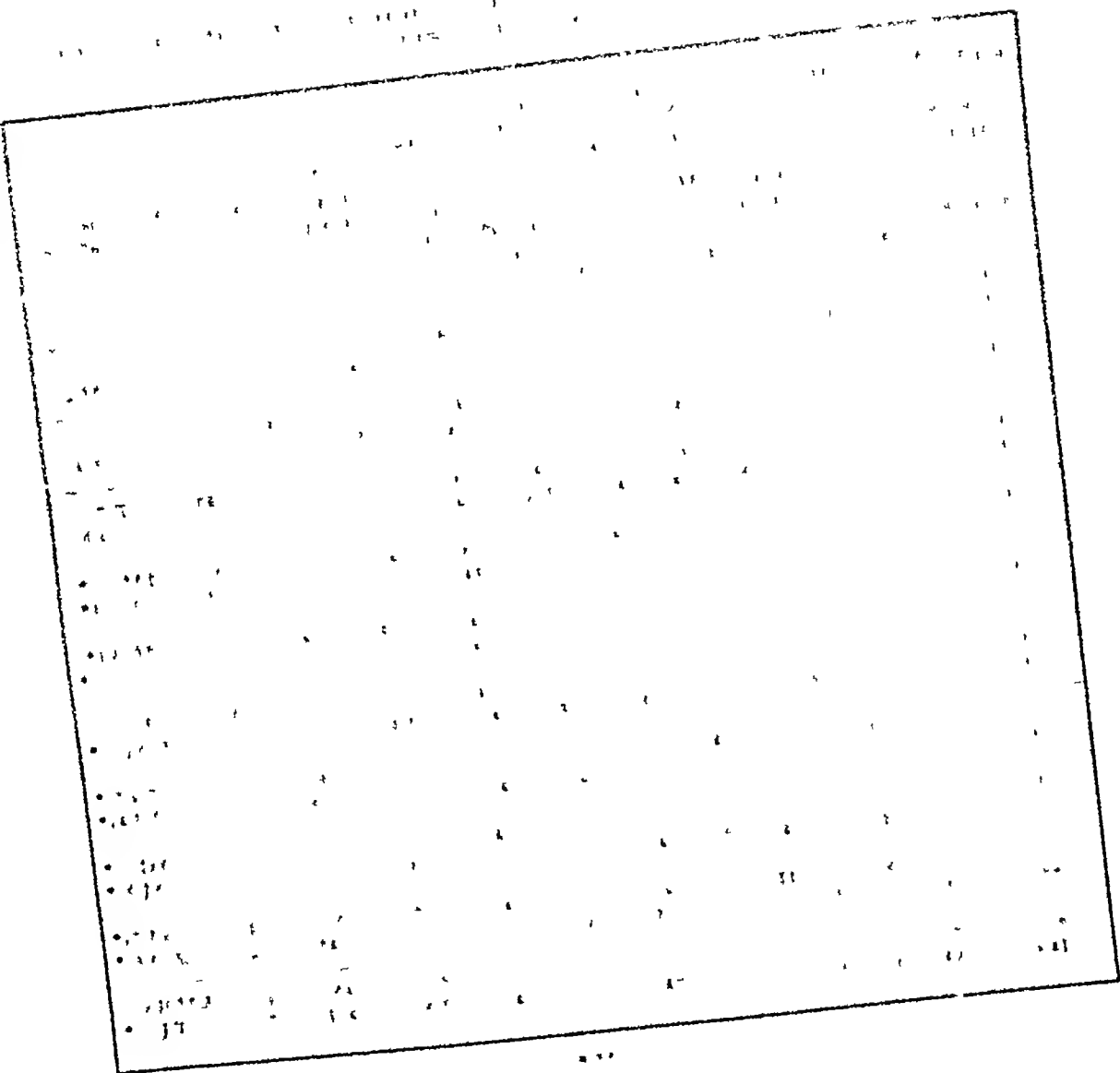
Of the twenty patients in Group I who with three exceptions were all followed for 24 months after treatment ceased, fifteen became free of circulating microfilariae. Of the five who were not negative, two had very low parasite counts which appear likely soon to drop to zero the three others, however all of whom had had negative blood samples at some time during the observations still had parasites in their blood stream and presented good evidence either of re infection or else of relapse of the old infections. One of these three subjects however had received only 1.3 grammes of drug in 9 days—the lightest treatment given any patient.

Of the ten patients in Group II all of whom were followed for 14 months from the end of the second course of therapy seven are now negative, and the remaining three have comparatively few parasites. Of the five patients in Group III followed for 16 months, three are now negative and two have sharply reduced numbers of microfilariae.

Taken altogether of thirty five patients who were treated with neostibosan twenty-five appeared to have been freed of infection with *W. bancrofti*, and five others appeared likely soon to be free of the infection. In the remaining five individuals, the drug, as applied, had less than a fully curative action.

#### NEOSTAM (Burroughs Wellcome).

Eleven patients were intensively treated with neostam, from 5.8 to 11.4 grammes of drug being administered to most patients in 14 or 15 days. It was difficult to maintain the drug in most patients because of the severe reactions generally seen.



DATA ON TEN PATIENTS WITH FILARIASIS (Continued)

Case number	Age.	Weight (lb.).	Drug given (grammes).	Days of treatment.	First course of therapy			
					Number of microfilariae in 60 mm. bl. at designated times.			
					Before treatment.	At end of treatment.	Months after of treatment	
							2	6
21 ALN	12	74	6.5	46	37	84	16	12
22 ALE	16	102	6.9	40	126	126	111	46
23 H.R.R.	11	56	7.3	34	76	6	6	16
24 D.R.	13	76	6.1	40	72	120	72	38
25 C.F.	6	8.	6.4	32	152	93	90	109
26 C.D.	16	112	7.1	40	120	126	72	42
27 F.G.	12	71	6.1	40	630	624	245	217
28 L.O.	14	128	7.2	40	129	166	84	67
29 J.R.	16	85	6.6	32	184	129	138	61
30 B.B.L.	14	81	7.1	40	76	87	90	24

Males.

Five of the patients were apparently freed of microfilariae by the 13th month after treatment ended, and all other patients (except No. 11 J.M.O., who was followed for only 1 month) showed a sharp reduction in the number of parasites present. It appears likely that the blood of additional patients treated with neostam will become negative as more time elapses (see Table IV).

#### UREA STIBAMINE (E. R. Squibb and Sons, Brahmachari).

Two preparations of urea stibamine were used in this study—one being obtained from E. R. Squibb and Sons, New York, and the other from Brahmachari, India. The Squibb preparation was decidedly the less toxic material. Three patients were given approximately 7 grammes of the Squibb drug in 17 days. Three patients on the Brahmachari drug received from 3.3 to 4.9 grammes within the same period.

After 16 months, the bloods of four patients were negative and that of the remaining two individuals had comparatively few microfilariae. No

II

*bancrofti*) GIVEN TWO COURSES OF NEOSTIBOSAN

Second course of therapy								Microfilaria level, per cent change, entire period of observation
Drug given (gramme)	Days of treatment	Number of microfilariae in 60 c mm blood at designated times						
		Before second course	At end of second course	Months after end of treatment				
				1	5	8	14	
7.0 12.5	10 14	13 65	1 11	2 16	0 0	0 0	0 0	—100 —100
5.0 11.5	9 15	16 67	1 9	0 13	1 4	0 0	0 0	—100 —100
9.0 12.0	11 14	65 31	0 21	0 11	0 15	1 2	0 0	—100 —100
11.1 12.0	15 14	284 71	60 57	70 41	35 15	15 4	0 1	—100 —99
10.0 12.5	14 14	69 75	39 40	41 63	28 41	25 18	2 18	—99 —76

difference in effectiveness was seen between the two varieties of urea stibamine (See Table V)

#### STIBANOSE (Winthrop Chemical Company)

Only five patients were treated with stibanose, these persons being given 13.8 to 15.2 grammes of drug (in 6.67 per cent solution) in 11 to 13 days. No reactions to this drug were seen in any patient at any time.

Twelve months after treatment ceased the blood of one individual was negative for microfilariae and that of one other had very few parasites. The three remaining patients, however, all still presented essentially the same number of parasites as that originally seen. (See Table VI)

#### FLUIDIN (Winthrop Chemical Company)

Fifteen patients were treated with fluidin, most of these being given a total of between 3.5 and 6.1 grammes of drug (in 6.3 per cent solution) in from 11 to 14 days. Fluidin as administered was not well tolerated by the



TABLE III.

DATA ON FIVE PATIENTS WITH FILARIAE (*Wuchereria bancrofti*) GIVEN A MODERATE INTENSIVE COURSE OF MOUTONIAN.

Case number	Age.	Weight (lb.).	Drug given (gramme).	Period of treat ment (days).	Showing the number of microfilariae in 60 cmm. of blood from treated patients at designated times.								Macrofilaria level, per cent change entire period of observation.
					Before treat ment.	At end of treat ment.	Months after end of treatment.						
							1	3	6	9	12		
21 E.G.	8	48	11.4	18	30	0	0	8	0	8	0	-100	
22 M.M.	14	76	9.8	14	92	12	38	3	0	0	0	-100	
23 J.A.A.	21	134	18.8	13	70	20	10	9	3	0	0	-100	
24 V.N.	11	68	11.4	18	621	180	267	224	187	90	29	-84	
25 C.B.L.	26	162	13.3	12	104	144	94	24	93	82	80	-68	

All are males.

TABLE IV.

DATA ON ELEVEN PATIENTS WITH FILARIAE (*Wuchereria bancrofti*) TREATED WITH MOUTONIAN.

Case number	Age	Weight (lb.)	Drug given (gramme)	Period of treat ment (day)	Showing the number of microfilariae in 60 cmm. of blood from treated patients at designated times						Microfilariae level, per cent. change nature period of observation.
					Before treat ment.	At end of treat ment.	Months after end of treatment.				
							1	8	7	12	
1 A.L.B.	23	107	10.6	14	92	24	91	—	8	0	—100
2 B.M.R.	23	113	10.6	14	366	121	74	123	8	8	—100
3 F.T.	23	140	7.7	18	180	175	48	8	8	0	—100
4 L.M.S.	26	113	9.9	14	94	34	40	4	12	0	—100
5 J.M.G.	21	115	9.8	18	83	33	120	48	4	8	—100
6 A.A.R.	18	127	11.0	14	35	8	29	21	11	1	—87
7 R.N.V.	23	178	11.4	14	319	25	90	0	0	10	—96
8 A.F.	25	148	2.1	8	33	—	19	18	13	8	—78
9 C.B.C.	23	134	18.8	14	1	4	—	4	4	8	—71
10 A.V.G.	31	121	18.1	14	290	49	155	—	118	118	—80
11 J.M.O.	19	148	8.7	18	165	47	187	—	—	—	+12

All are males.

TABLE V

DATA OF SIX PATIENTS WITH *Huchereria bancrofti* TREATED WITH UREA STIRAMINE.

Case number	Age	Weight (lb.)	Drug given (gramme)	Period of treatment (days)	Showing the number of microfilariae in 60 c mm of blood from treated patients at designated times							Microfilaria level per cent change entire period of observation
					Before treatment	At end of treatment	Months after end of treatment					
							1	3	6	10	16	
*1 R C A	25	140	0.8	16	3	4	0	0	0	0	0	-100
*2 J I I	10	141	7.1	17	20	5	2	0	0	0	0	-100
3 J H M	10	120	4.2	14	12	0	0	0	0	—	0	-100
4 M I F	20	127	7.3	11	14	18	6	0	0	—	—	-100
5 H M	21	109	4.0	16	334	96	163	153	163	56	10	-97
*6 A I A	30	141	7.1	17	155	181	200	115	128	161	16	-89

All are males

\* Given drug made by F. R. Squibb and Sons, others given Brahmachari preparation

TABLE VI

DATA OF FIVE PATIENTS WITH THIASIAS (*Huchereria bancrofti*) TREATED WITH STIRAMINE

Case number	Age	Weight (lb.)	Drug given (gramme)	Period of treatment (days)	Showing the number of microfilariae in 60 cmm of blood from the patient at designated time						Microfilaria level per cent change entire period of observation
					Before treatment	At end of treatment	Months after end of treatment				
							3	5	9	12	
11 V	—	40	1.8	11	—	53	—	0	0	0	-100
12 B	1	102	14	17	4	—	—	18	—	8	-82
13 D	1	4	1.2	17	120	—	—	1	1	118	-99
14 S I	15	—	1.2	1	45	—	4	45	—	1	-98
15 S I	12	—	1.2	1	1	—	—	—	—	118	-99

patients. Drug had to be omitted frequently in most patients, and was suspended entirely in some cases because of severe reactions.

Fifteen months after treatment ended, three patients were apparently free of microfilariae, and all others had decidedly fewer parasites in their blood than before treatment. (See Table VII)

TABLE VII

DATA ON FIFTEEN PATIENTS WITH FILARIASIS (*Wuchereria bancrofti*) TREATED WITH SPECIA.

Case number	Age.	Weight (lb.).	Drug given (grammes).	Period of treatment (days).	Showing the number of microfilariae in 100 c.mm. of blood from treated patients at designated times.								Microfilariae level, per cent. change, entire period of observation.
					Before treatment.	At end of treatment.	Months after end of treatment.						
							1	4	8	12	18		
1 J.J.	39	124	2.7	11	148	44	68	81	2	14	8	-100	
2 S.V.A.	31	144	4.6	13	257	132	184	117	19	11	8	-100	
3 S.N.R.	18	122	5.3	13	112	81	18	58	23	8	—	-100	
4 G.L.F.	18	123	4.8	14	184	40	53	27	8	3	1	-98	
5 B.A.C.	21	140	3.5	14	273	33	103	78	83	—	1	-92	
6 E.M.C.	27	116	4.4	11	64	34	28	28	—	4	1	-83	
7 R.R.Z.	21	120	3.3	13	181	44	171	—	81	12	8	-87	
8 J.R.C.	18	123	1.4	8	289	123	280	190	18	—	—	-84	
9 L.F.C.R.	22	126	4.2	13	289	29	99	18	58	24	27	-83	
10 P.R.D.	28	169	8.4	11	233	18	10	8	29	23	14	-80	
11 G.G.	30	113	8.1	14	334	31	141	87	88	84	30	-87	
12 A.R.H.	23	126	4.2	13	323	28	480	163	315	148	128	-83	
13 S.B.P.	22	122	4.2	13	633	44	218	—	88	—	—	-78	
14 J.R.A.	18	127	5.0	14	517	181	608	—	240	84	203	-80	
15 S.R.M.	24	123	1.4	7	323	78	218	174	—	—	—	-23	

All are males.

#### ANTHOMALINE (Specia Merck and Company).

Twenty patients were treated with anthomaline ten of these being given the Specia product and ten the Merck preparation. The Specia drug was given over usually from 17 to 20 days, most patients receiving from 3 to 3.6 grammes of drug (in 8.0 per cent. solution). From 2.3 to 4.2 grammes of the

Merck drug were given to most subjects over from 11 to 13 days. Anthiomaline was not well tolerated and its administration had to be suspended in several patients.

Of the ten individuals treated with anthiomaline (Specia), four had no microfilariae in their bloods after 15 months and five others all presented far fewer parasites than before treatment\*. The remaining patient was seen only during 1 month after treatment ceased and, during this time, essentially no change occurred in his microfilaria count (See Table VIII).

TABLE VIII  
DATA ON TEN PATIENTS WITH FILARIASIS (*Wuchereria bancrofti*) TREATED WITH ANTHIOMALINE (Specia)

(See Table VIII), essentially

TABLE VIII

TEN PATIENTS WITH FILARIASIS (*Wuchereria bancrofti*) TREATED WITH ANTHIOMALINE (Specia)

Case number	Age	Weight (lb)	Drug given (gramme)	Period of treatment (days)	Showing the number of microfilariae in 60 c mm of blood from treated patients at designated times							Microfilaria level, per cent change, entire period of observation	
					Before treatment	At end of treatment	Months after end of treatment.						
							1	3	7	11	15		
*1 VG *2 C.A.C.	17 18	118 106	3 1 2 3	17 16	47 33	3 4	3 4	1 —	0 0	— 0	0 0	—100 —100	
3 FVB 4 BCR	22 23	139 133	3 0 3 6	17 20	433 289	199 15	133 56	54 44	0 47	0 —	0 0	—100 —100	
5 PF 6 JLT	18 20	111 111	2 4 3 6	14 20	951 677	180 132	528 397	344 356	166 212	50 99	6 143	—99 —78	
7 RRM 8 GSM	22 35	124 107	3 6 3 6	20 20	1,094 722	134 163	641 438	536 334	489 60	248 216	158 276	—76 —61	
*9 GG 10 DVG	15 22	143 124	1 1 3 0	7 18	139 22	115 10	113 17	67 —	36 —	32 —	45 —	—60 —22	

• Females

Of the ten subjects given anthiomaline, 9 had microfilariae after 14 months. 1 could not be examined.

\* Females

Of the ten subjects given anthiomaline (Merck), three were negative for microfilariae after 14 months and all others, except one (No 10, L R S), who could not be followed, had sharply reduced numbers of microfilariae (See Table IX).

TARTAR EMETIC (Abbott Laboratories)

Only four patients were treated with tartar emetic. Because of a severe

\* Essentially similar results have been reported by BROWN (1945), using this drug

TABLE IX.

DATA ON TEN PATIENTS WITH FILARIAE (*Wuchereria bancrofti*) TREATED WITH VEDONALINE (Merck)

Case number	Age.	Weight (lb.).	Drug given (gramme).	Period of treatment (days).	Showing the number of microfilariae in 100 cmm. of blood from treated patients at designated times.							Microfilariae level, per cent. change, entire period of observation.
					Before treatment.	At end of treatment.	Months after end of treatment					
							1	2	7	11	14	
1 P.P.P.	30	146	2.2	12	74	0	3	8	0	0	0	—100
2 E.O.C.	20	125	4.5	12	89	5	48	1	0	0	0	—100
3 M.A.D.B.	19	153	2.0	12	317	172	234	17	1	0	0	—100
4 P.R.R.	26	126	2.7	9	274	104	107	61	47	2	5	—84
5 F.F.	21	129	1.6	6	245	244	173	142	—	5	11	—96
6 M.A.S.R.	27	130	2.0	12	99	72	1	—	—	8	4	—91
7 M.L.R.	21	112	2.2	12	123	111	63	—	30	—	23	—75
8 S.O.C.	22	106	2.7	12	163	52	136	73	108	29	44	—73
9 H.G.C.	12	126	1.6	11	263	270	282	172	241	23	165	—44
10 L.R.S.	18	129	2	12	1024	547	—	—	—	—	—	—12

All are males.

reaction to drug in one subject early in the course this drug was never applied intensively. All patients were given only between 0.7 and 0.9 gramme of drug in 1 per cent. solution in 14 days. All of the four patients showed a decided reduction in microfilariae in 13 months after treatment ceased, but no individual was negative for the parasites (See Table X.)

## MELARSEN OXIDE (Parke Davis and Company).

As stated earlier some subjects were treated with melarsen oxid orally and others by vein. Three patients received the drug orally in 50 mg. capsul three times daily. Altogether the three persons received 1.05, 1.2 and 1.5 grammes of drug in 8, 13 and 14 days respectively. The remaining fifteen persons were given a total of 0.03 to 0.09 gramme of the drug dissolved in propylene glycol intravenously over from 7 to 9 days. Although most patient did not react to the drug in any way whatever some individuals manifested severe responses. Two subjects developed arsenical encephalitis and were gravely ill for a week or more. Fortunately both patients recovered, although one continued to show partial right side paralysis for at least 12 months.

TABLE X.

DATA ON FOUR PATIENTS WITH FILARIASIS (*Wuchereria bancrofti*) TREATED WITH TARTAR EMETIC

Case number	Age	Weight (lb )	Drug given (gramme)	Period of treatment (days)	Showing the number of microfilariae in 60 c mm of blood from treated patients at designated times							Microfilaria level, per cent change, entire period of observation
					Before treatment	At end of treatment	Months after end of treatment					
							1	3	6	10	13	
1 RCF	37	140	0 79	14	174	120	174	157	69	0	9	—94
2 EPD	27	140	0 73	14	215	177	257	108	39	13	49	—77
3 MJF	21	121	0 77	14	65	45	46	13	0	0	15	—76
4 RDM	25	137	0 88	14	281	267	208	83	172	30	113	—59

All are males

One of the patients treated orally lost all microfilariae from his blood, but the remaining two persons still presented parasites, although in greatly reduced numbers, 14 months after treatment ceased. Of those treated by vein, seven were negative for microfilariae and, after 13 months, all others had substantially fewer microfilariae than before treatment (See Table XI)

#### MICROFILARIAE IN LARGE (10 c c) SAMPLES OF NOCTURNAL BLOOD

From forty-five patients—for all but nine of whom the 60 c mm blood samples had become free of microfilariae as the result of treatment—10 c c of nocturnal blood were drawn and carefully searched for the parasites by the samples, thirty were also negative by the 10 c c sample. The remaining six patients, who were positive only in the 10 c c samples, presented only one, method described above. Of the thirty-six patients with negative 60 c mm two, two, twenty-one, twenty-two and twenty-seven microfilariae, respectively, in this large volume of blood. As would be anticipated, the nine patients who were positive by the 60 c mm blood sample, all presented many microfilariae in the 10 c c of blood. The data on this part of the study are shown in Table XII.

#### RELATIONSHIP OF THE ANTIMONY PLASMA LEVEL TO THE DISAPPEARANCE OF MICROFILARIAE

From representative patients, blood samples were collected and the plasma level of antimony determined. It was thought possible that some correlation

TABLE XI.

DATA ON EIGHTEEN PATIENTS WITH FILARIASIS (*Wuchereria bancrofti*) TREATED WITH MELLARIN OXIDE

Case number	Age.	Weight (lb.).	Drug given (grams).	Period of treatment (days).	Showing the number of microfilariae in 50 c.mm. of blood from treated patients at designated times.							Microfilaria level, per cent change, entire period of observation.
					Before treatment.	At end of treatment.	Months after end of treatment.					
							1	3	7	10	12	
1 P.R.M.	19	143	1.50	14	9	13	4	0	0	—	—	—100
*2 M.L.A.L.	23	127	1.65	8	82	12	16	14	13	3	4	—85
*3 S.J.	23	129	1.20	13	409	537	302	413	238	94	133	—67
4 L.F.S.D.	19	117	0.09	9	10	—	0	0	—	—	—	—100
5 V.C.H.	22	130	0.04	9	1.3	0	0	—	0	0	0	—100
6 P.R.C.	22	141	0.04	7	29	33	10	1	0	0	0	—100
7 M.O.P.	22	149	0.08	9	51	79	43	0	1	0	0	—100
8 H.B.	22	129	0.03	7	8	47	20	—	3	9	0	—100
9 R.L.	25	118	0.09	9	31	12	47	4	0	1	0	—100
10 T.C.	24	135	0.08	9	51	39	32	—	30	—	0	—100
11 M.C.D.	4	147	0.05	7	418	484	319	25	285	17	—	—83
12 F.M.C.	23	114	0.09	9	39	58	23	1	19	—	4	—96
13 L.E.O.	22	126	0.09	9	8	24	9	—	0	0	1	—57
14 J.R.P.	30	123	0.06	7	99	141	134	37	77	—	20	—78
15 R.F.B.	20	148	0.05	7	108	149	97	28	49	17	24	—45
16 M.A.B.	24	148	0.06	7	79	136	119	70	2	11	23	—57
17 V.C.S.	4	131	0.04	7	1 123	1,000	573	886	479	—	—	—37
19 A.G.C.	21	119	0.04	7	142	109	179	93	—	—	—	—31

All are males.

Treated orally

might be shown between the effectiveness of a given drug and the blood level attained during its administration. All samples were obtained at approximately 8 a.m. of the day following the last injection of drug. D-determinations were obtained for nine patients under intensive therapy with neosubosan, six with neostam, one with stibanose, twelve with anthomaline, eight with fuadin, and four with tartar emetic. No samples were taken from patients treated with urea stibamine. All of the antimony determinations were made by Dr. ALFRED







A



B

SECTION OF NODULE SURGICALLY REMOVED FROM THE SCROTAL SAC OF PATIENT NO 3 AC, WHO HAD BEEN TREATED WITH FUMIGY SHOWING REACTION ABOUT FILARIAL WORM.

A. Section of entire nodule. ( $\times 5$ )

B. Arthus-like appearance of reaction about worm. ( $\times 50$ )

TABLE XII  
COMPARATIVE NUMBERS OF MICROFILARIAE SEEN IN 60 C MM AND IN 10 C C SAMPLES  
OF NOCTURNAL BLOOD FROM SELECTED PATIENTS

Drug	Patient	Months after treatment ended	Months since microfilariae were last seen	Microfilariae in 60 c mm blood before treatment	Microfilariae in blood when last seen	
					In 60 c mm	In 10 c c
Neostibosan	1 LET			3		
	2 MR	24	24	9	0	0
	3 VR	24	24	24	0	0
	4 AEB	24	24	15	0	0
	6 CIR	24	21 5	216	0	0
	7 EMD	24	18	18	0	0
	10 CP	24	15	24	0	0
	11 CAR	24	15	231	0	0
	12 J.O.A.	24	12	82	0	0
	13 PB	24	9	177	0	0
	21 MN	24	9	27	0	0
	22 ME	14	6	136	0	0
	23 HRR	14	9	18	0	0
	24 DR	14	9	72	0	0
	25 CF	14	6	123	0	0
	26 CD	14	6	120	0	22
	27 FG	14	3	530	0	0
	31 EG	14	3	30	0	0
	32 MM	16	3	93	0	2
	33 J.A.A.	16	13	79	0	0
Neostam	18 OA	16	7	30	0	1
	20 JT	24	—	27	8	0
	38 IO	14	—	120	105	180
Urea stibamine	1 ALB	13	7	62	0	2
	2 MMR	13	3	366	0	0
	7 RNV	13	—	249	10	61
Stibanose	1 R.C.A.	16	16	3	0	0
	2 JFL	16	13	20	0	0
Fuadin	2 JBM	16	16	12	0	0
	1 JV	12	7	32	0	0
Anthiomaline (Specia)	4 GMF	15	—	154	1	86
	1 VG	15	8	47	0	0
	2 C.A.C.	15	8	33	0	0
Anthiomaline (Merck)	3 FVB	15	3	433	0	0
	1 PPP	14	7	74	0	0
	2 EGC	14	7	99	0	0
Tartar emetic	3 MADB	14	7	317	0	21
	4 PRR	14	7	224	5	0
Melarsen oxide	1 RCF	13	—	174	9	527
	3 MJF	13	—	65	15	462
	5 VCH	13	13	125	0	0
	6 PBC	13	7	20	0	0
	7 MOP	13	10	51	0	0
	8 HB	13	10	8	0	27
	9 RL	13	3	31	0	0
	13 LEO	13	—	14	1	56

GELLHORN Department of Pharmacology College of Physicians and Surgeons, using his modification of the Rhodamine B method (GELLHORN in press). The complete data obtained from these determinations have been presented elsewhere by GELLHORN *et al* (1947), but a general summary sufficient for present needs, follows.

Accurate determinations of antimony were possible only in patients given neostibosan and neostam. In blood sample from all patients given other drugs, no more than "traces" of antimony were detected, traces being defined as less than 0.05 mg of antimony per 100 c.c. of plasma. The plasmas of the nine neostibosan and of the six neostam patients contained the amounts of antimony indicated in Table XIII.

TABLE XIII.

DATA ON SELECTED PATIENTS SHOWING THE ANTIMONY PLASMA LEVELS MEASURED BY THE END OF TREATMENT AND THE PERCENTAGE CHANGE IN MICROFILARIA LEVEL DURING THERAPY

Drug.	Patient.	Antimony in plasma at end of therapy (mg. per 100 c.c.).	Percentage change in microfilaria level during therapy
Neostibosan	M.M.	1.47	-87
	E.G.	1.18	-100
	V.N.	1.11	-79
	J.A.A.	1.08	-87
	D.R.	1.06	-86
	F.G.	0.88	-78
	C.D.	0.87	-82
	C.M.	0.73	-25
	I.O.	0.48	-19
Neostam	FT	0.18	-7
	J.A.G.	0.17	-34
	J.M.O.	0.14	-71
	R.N.V.	0.10	-80
	A.A.R.	0.10	-77
	M.A.L.R.	0.05	-66

It is difficult to draw conclusions from Table XIII as to a possible correlation between high antimony plasma levels and the disappearance of microfilariae from the blood. Yet, in the neostibosan group patients I.O., C.M., and C.D. with the three lowest plasma levels of antimony showed the smallest percentage declines in microfilariae and patients E.G. and V.N. with the two highest antimony plasma levels, had the largest decline in circulating parasites. Others in the group, as well as all those in the neostam group, do not show more than a moderate decline in microfilariae.

levels in the plasma lost most of their microfilariae (and, in some cases, all of them) during the course of therapy

### EFFECT UPON ADULT WORMS

In most patients, no effects were seen which could be interpreted as indicative of a deleterious action by drug upon the adult filarial worms. In a few of the adult male patients who were treated intensively, however, one or two tender nodules ranging up to 1 cm in diameter, appeared in the scrotum late in the course of therapy or during the 1st week or 10 days after treatment ceased. One nodule was surgically removed from a patient by Dr J. S. COLON, Urologist of University Hospital, San Juan, and sections of this nodule were prepared and studied by Dr E. KOPPISCH, Pathologist of the School of Tropical Medicine. The sections revealed a filarial worm surrounded by an extensive inflammatory area suggestive of an Arthus reaction. From the condition of its ovarian nuclei, the worm was considered to be recently dead (see Plate A and B). Photomicrographs of a section of this nodule appeared in one of the earlier reports of this work (CULBERTSON, ROSE, HERMANDEZ-MORALES *et al.*, 1946). Similar painful nodules found in the scrotal sac of some other intensively treated patients were not removed but were kept under careful observation. In all cases, the reaction subsided within 2 weeks, the nodules regressing in size thereafter to become tiny hard granules which, in some, persisted for several months. No permanent impairment has yet become evident in any patient. The reactions were observed in patients treated with various antimony compounds as well as in one individual given the arsenical preparation

### OBSERVATIONS IN UNTREATED CONTROL PATIENTS

The untreated control patients were all students between 8 and 17 years old at a boys' home near San Juan. None showed symptoms of filariasis although all had microfilariae in their nocturnal blood. Their bloods were examined at intervals over 26 months. Microfilariae were invariably found in the nocturnal blood of every patient at every examination. In some patients, the number of parasites increased sharply during the course of the observations, presumably because microfilariae were gradually accumulating in the blood as the infection progressed. The data on the control group appear in Table XIV.

### DISCUSSION

The data in this paper indicate that infection with *W. bancrofti* can be eradicated from patients by the administration of any of several antimony-arsenic-containing compounds. Drug generally must be given in comparatively high dosage, and reactions—particularly with certain compounds—are often severe if not serious. Presumably the drugs act chiefly on the adult worms, as microfilariae often persist, though in gradually diminishing numbers, for many months after treatment has ceased. No effects have yet been seen which

TABLE XIV

OF FIFTY-ONE UNTREATED CONTROL PATIENTS WITH FILARIAE (*Wuchereria bancrofti*).

Case number	Age.	Weight (lb.).	Number of microfilariae in 20 mm. blood at designated times.							Microfilariae lev. 1, per cent. change, entire period of observation.
			When first seen	Months after first examination.						
				1	6	12	17	20	24	
1 L.B.	14	60	125	153	162	143	86	161	56	-30
6 G.M.	14	96	36	60	38	51	40	42	29	-44
6 F.V.	12	62	6	6	6	6	7	7	4	-33
4 V.BLO	12	66	209	—	322	239	219	—	—	+6
5 M.R.	14	82	360	222	218	569	232	562	418	+16
6 V.G.	12	78	27	21	36	22	43	80	23	+29
7 M.R.	12	80	616	223	223	97	47	—	—	+59
6 L.T.	17	115	27	—	61	62	41	23	44	+62
6 F.N.	12	65	45	21	27	18	1	50	76	+72
10 S.R.	14	124	62	6	22	27	8	29	116	+89
11 J.F.	16	120	6	6	6	12	7	—	—	+123
12 J.M.	2	67	67	—	176	286	272	234	224	+157
12 V.B.	14	102	0	6	16	12	9	—	—	+222
14 F.V.	14	66	97	31	56	122	—	11	—	+616
16 J.R.	2	66	6	—	18	24	22	60	66	+1906

All are males.

indicate that the killing of adult filarial worms through therapy leads to symptoms suggestive of elephantiasis or to other impairment of the patient.

The authors do not consider that any of the drugs used thus far represent an ideal therapeutic agent for bancroftian filariasis. The disease is of non fatal character and, presumably symptoms although serious, develop only in relatively few of those who harbour the infection. Because with all the drugs thus far tried (except stibanose), more or less severe reactions can be expected, therapy of this form of filariasis should be undertaken only after the most careful consideration, lest the treatment prove more hazardous to the patient than the disease itself.

Of the various drugs used, neostibosan was the most satisfactory. It manifested a high order of activity on the infection and was well tolerated by patients. Stibanose was the best tolerated of all the drugs used, but this

substance had but slight effect upon the parasites. The trivalent antimony compounds in general were poorly tolerated and the single arsenical, though effective, induced serious reactions in some patients with little or no warning.

### SUMMARY

One hundred and twenty-nine Puerto Rican patients have been included in the present experimental study of the chemotherapy of *bancrofti* filariasis. All of the persons harboured microfilariae of *W. bancrofti* in their nocturnal bloods, but only three of the group presented symptoms of the infection. Fifteen of the individuals (all free of symptoms) have been kept untreated as controls on the infection and on the effects of treatment. The remaining 114 persons have been treated with one of eight compounds of either antimony or arsenic. Generally treatment was carried on intensively and, with some drugs, had to be suspended in certain patients because of the severity of reactions.

In Table XV below are given the names of the eight drugs used, the number of patients treated with each drug, and the number of patients given each drug from whom all microfilariae have disappeared.

TABLE XV,

Drug	Number of patients treated	Number of patients freed of microfilariae
Neostibosan	35	25
Neostam	11	5
Urea stibamine	6	4
Stibanose	5	1
Fusadin	15	3
Anthiomaline	20	7
Tartar emetic	4	0
Melarsen oxide	18	8

Of the fifteen untreated control patients, all have continued to show microfilariae throughout the period of observations.

It is concluded that *W. bancrofti* can be eradicated from many patients by the administration of any of several compounds of antimony or arsenic. Of the compounds thus far tried, the pentavalent antimonial neostibosan has shown most promise as a practical therapeutic agent because it is well tolerated by patients and has a marked effect against the parasites.

### III. CHEMOTHERAPY OF *LOA LOA* INFECTIONS IN MAN

(In collaboration with HARRY M. ROSE)

The speaker has had but little to do with the treatment of the *Loa* infections about to be described, his contribution amounting to little more than

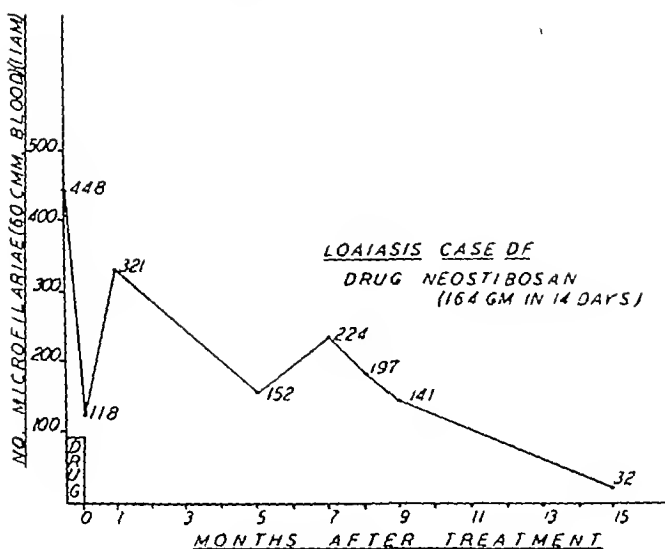
performing a part of the counts of blood parasites. The work was designed and carried on essentially in its entirety by Dr H. M. Ross.

Three patients with loiasis came under our supervision in New York City in September 1945. All were members of the same family group (husband, wife and daughter) who had returned a few weeks earlier after residence as missionaries for several years in French Equatorial Africa. The husband (Case D F), age 40 had many embryos of *Loa loa* in his day blood when first seen—for example, 448 microfilariae were counted in 60 c.mm. of finger blood taken at 11 a.m. on 14th September 1945 just before treatment was instituted. He had previously suffered only occasionally from Calabar swellings. The wife aged 37 had no microfilariae in her blood but had been troubled on many earlier occasions with Calabar swellings, one such swelling appearing during the course of the treatment subsequently given. The daughter age 9 had no microfilariae of *Loa loa* in her blood, but had been troubled almost constantly for several years with Calabar swellings at times the distress from them becoming so acute that she could not attend school. The three patients were admitted to hospital in September and October 1945 where they were given intensive therapy with neostibosan for a period of 2 weeks, the husband, wife, and daughter respectively receiving 16.4, 11.1 and 9.4 grammes of the drug in the 14 days. All tolerated the drug quite well, the husband accepting during most of his course 1.5 grammes of drug daily with no untoward effects. The daughter however experienced some nausea at various times and several of her scheduled injections had to be omitted.

The Chart shows the numbers of microfilariae counted at the indicated times in 60 c.mm. samples of the peripheral blood. It will be seen that, after 15 months, 92 per cent. of the subject's microfilariae had left his blood. During the interval since treatment, the patient has been free of all symptoms of the infection. The remaining two patients both improved markedly with respect to symptoms soon after being treated and neither has shown Calabar swellings in the last 6 months (to January 1947).

Unfortunately the work on loiasis has no control because only few cases of the infection appear in New York. Since our experience with this disease is limited largely to those few patients here described we cannot by our the reduction in microfilaria level seen in one subject and the improvement in symptoms noted in the other two individuals resulted from chemotherapy. It is possible these cases would have shown similar changes without treatment, by reason of having moved to a non-endemic zone. Nevertheless, I have thought it useful to place the data before you, without conclusions, for whatever they may be worth.

An extended report from the work with the loiasis patients will be prepared by Dr Ross for publication elsewhere.



CHART—Showing the number of microfilariae in 60 cmm of finger blood taken at 11 a m before treatment, at the end of the period of treatment, and at the designated months after therapy ceased. The patient received 164 grammes of neostibosan during 14 days.

#### IV CHEMOTHERAPY OF *ONCHOCERCA VOLVULUS* INFECTIONS IN MAN

(In collaboration with HARRY M. ROSE, LUIS FIGUEROA ORTIZ, FRANCISCO RUIZ REYES, and ROBERTO NETTEL.)

Because of the apparent success in treating cases of *W. bancrofti* infection with the various drugs, it was decided to attempt a similar study in onchocerciasis. For this work the pentavalent antimony compound neostibosan was used, since in the earlier parts of this study it had been well tolerated by patients and had proved comparatively effective upon the related parasites. After arrangements had been made through Dr. MANUEL MARTINEZ-BAEZ, Under-Secretary of Health in the Mexican Government, and Dr. JOSÉ ZOZAYA, Director of the Institute of Tropical Diseases in Mexico City, work was begun in February, 1946, in the Government Hospital for Onchocerciasis Investigations at Huixtla, Chiapas, Mexico.

##### THE PATIENTS

Altogether, forty patients infected with *Onchocerca volvulus* were included in the present study. All were native Mexicans of mixed race who contracted their infections while resident on upland coffee plantations in the mountains near Huixtla. Some had resided in the hospital since it was first opened,



approximately 5 years earlier and practically all had been hospitalized for at least 1 year. Since Huixtla is not itself a centre of infection none of the patients, probably had been re-infected during the period of hospitalization. The patients ranged from 5 to 63 years of age. Practically every patient had more or less severe ocular pathology and three were blind from the *O. colubus* infection. Many showed the distinctive facial pigmentation (*mal morado*) often seen in this disease as it occurs in Mexico. Some cases also exhibited a rather characteristic oedema of the cheeks. Many had nodules formed about the mature parasites in various parts, and practically all individuals at some time during hospitalization had had nodules removed surgically. Except for their infections with *O. colubus* the patients were all considered to be essentially in good health.

*Administration of the Drug*—The whole number was divided into two equivalent groups of twenty patients each, one group being intensively treated with neostibosan for 2 weeks and the other being kept untreated as control. The treated group of patients received drug twice daily (8 a.m. and 5 p.m.) a total of from 11 to 13 grammes of neostibosan being given in 14 days, the total daily dose on most days after the second being 1 gramme. All injections were made by vein.

*Tolerance of Drug*—All the patients tolerated the drug particularly well for the first 10 days, none of them showing even the nausea or vomiting which had sometimes been observed in other subjects previously treated with the drug in Puerto Rico and New York. On the 11th day and thereafter till the end of treatment, however several of the patients experienced low grade fever and showed signs of general malaise. The effects were not considered to be significant, however until the last (14th) day of treatment. On that day several patients appeared frankly ill and injections were omitted from all who were in any respect indisposed. For several days after treatment ended, five of the twenty treated patients were confined to their beds and showed evidence of renal damage. All were very seriously ill and one of the group, unfortunately succumbed on the 3rd day after treatment ceased. Others slowly improved. The fear which gripped all the treated patients as a result of the death of one caused somewhat more than half of the treated group to leave the hospital at once and no further observations on these individuals have been possible. It is difficult to explain why the Mexican patients failed to tolerate the drug as administered since neostibosan had caused no significant reaction in patients previously treated in Puerto Rico and New York. There was little or no doubt, however that the effects were caused by the drug since none in the control group became ill.

#### ESTIMATION OF THE LEVEL OF INFECTION.

The comparative level of infection with *O. colubus* in various patients and at successive times in the same patient was determined by counting the

numbers of microfilariae which emerged from small pieces of skin removed from each cheek and from each side of the neck. An effort was made to remove approximately the same quantity of skin in each biopsy, and skin was removed from the same sites in all patients irrespective of the presence or absence of nodules enclosing parent worms. The tissue of the skin specimen was teased in an individual drop of salt solution on a microscope slide and this fluid was then searched under sixty times magnification for the presence of microfilariae. The number of microfilariae finally recorded for each patient represented the total number which emerged from the tissue obtained from the four sites.

### RESULTS

It has been possible to follow seven of the treated patients for 10 months since treatment ended and one other treated patient for 5 months. The microfilaria counts obtained before treatment, at the end of treatment, and at various times since treatment ceased, have been compiled for these eight individuals and are shown in Table XVI. Microfilaria counts obtained at various times on eight of the control patients who received no neostibosan are given in Table XVII.

TABLE XVI  
EFFECT OF NEOSTIBOSAN IN PATIENTS WITH *Onchocerca volvulus*

Patient number	Age	Weight (kg)	Amount drug (gramme)	Days of treatment	Number of microfilariae seen in biopsies from two cheeks and two sides of neck					Percentage change in microfilaria level over entire period of observation
					Be-fore treatment	At end of treatment	Months after treatment			
							1	5	10	
1 P L *†	23	45	12	13	64	7	2	1	2	-96
2 F P	20	64	13	14	148	14	35	42	25	-83
3 F G	8	19	13	14	11	1	5	2	—	-81
4 S D	32	40	11	13	050	40	360	231	190	-80
5 A G *†	10	23	12	13	127	49	55	20	48	-62
6 M G †	14	31	13	14	235	4	21	242	89	-62
7 I S *†	15	48	13	14	54	45	35	169	29	-46
8 J C	11	20	13	14	7	3	3	10	9	+28

\* Females

† One nodule removed 2 months after end of treatment

As the tables indicate, there appeared to be an immediate decline in the number of embryos in the skin of the treated patients. Within a month after the end of treatment, however, the decline had ceased and, in some patients, the number of microfilariae in the skin biopsies rose substantially toward what

it had been before the drug was given. During later months a slow secondary fall was noted in the microfilaria level, although it is impossible certainly to relate this decline to the treatment. Considering the high variation seen in the counts made on biopsies from the control patients not treated with neostibosan, it seems safest to ascribe little significance to the overall changes in microfilaria levels seen in the treated subjects. Since the drug had been given to the full limit of the patients' tolerance, the only conclusion possible from the work was that neostibosan given as described in a single intensive course did not produce a significant permanent effect in onchocerciasis—at least, within 10 months of the end of the course of therapy.

TABLE XVII.  
CONTROL PATIENTS WITH *Onchocerca volvulus*.

Patient number	Age	Weight (kg.)	Number of microfilariae in biopsies from two cheeks and two sides of the neck.				Percentage change in microfilaria level, entire period of observation.
			When first seen.	Months after first seen.			
				1	6	11	
21 M.S.	5	10	1	1	1	9	-100
22 E.R.	12	41	23	4	5	—	-91
23 P.G.	14	30	206	124	23	23	-83
24 J.D.†	8	10	0	2	1	—	-75
25 P.G.	41	43	149	802	800	123	-84
26 J.C.	9	10	0	2	1	11	+23
27 G.S.	9	19	31	49	19	48	+43
28 J.D.	12	23	31	49	34	84	+300

Females.

† One nodule removed 1 month after first seen.

## GENERAL SUMMARY

This paper offers data obtained from a fairly large project on experimental chemotherapy of several filarial infections. The first work, performed in cotton rats which naturally harbour a filarial parasite known as *Lilomorsoides carini*, showed definitely that this infection in this animal can be eradicated by the intensive administration of any of several antimony-containing drugs, these drugs having their principal action on the adult phase of the parasite. Methods essentially similar to those used in the experimental animals were then applied in infections with three human filarial worms—namely *W bancrofti*, *L. loa*, and *O. volvulus*.

In the work with *W bancrofti* 114 persons infected with this parasite were treated with any of several antimony-containing drugs, or with an arsenical, and fifteen additional patients were kept untreated as a control group. The

results obtained in the treated group varied considerably, depending upon the drug used. The most satisfactory drug, in respect both of the patients' tolerance and of the effect on the parasites, was neostibosan, a pentavalent antimony preparation. Of thirty-five subjects treated with neostibosan, twenty-five appeared entirely to lose their infections with *W bancrofti* within 2 years of the end of therapy. All the control patients remained infected for this period of observation, and some of these had substantially more intensive infections at the end of this time than at the beginning.

Only three subjects with *L loa* infection were available for experimentation. Of these, one had many microfilariae in his day blood, and the other two persons had marked symptoms (Calabar swellings) but no circulating microfilariae prior to therapy. After intensive treatment with neostibosan, the number of embryos fell by over 90 per cent. within 15 months in the blood-positive subject, and all symptoms in the other two individuals disappeared within 8 months after treatment. No control subjects on this part of the work were available.

Altogether, forty subjects with *O volvulus* were studied but, for reasons stated in the text, data on only sixteen can be reported. Of these sixteen, eight were treated intensively with neostibosan and eight were kept untreated as a control group. Ten months after therapy, no essential difference between the treated and control groups could be seen in the numbers of microfilariae in skin biopsies, although during the course of treatment, a marked reduction in the numbers of microfilariae in the skin seemed to occur. It was concluded that neostibosan is without permanent effect in onchocerciasis.

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## DISCUSSION

The President Ladies and gentlemen, I am sure you will agree with me that we have listened to a very interesting lecture on the chemotherapy of filariasis, and I should like to congratulate Professor CULBERTSON on the remarkably clear and lucid manner in which he has put his results before us. I am sure that many of those here will wish to ask questions, but before inviting them to do so I will ask Sir LEONARD ROGERS, who carried out some experiments on the treatment of filarians in India, to open the discussion.

Sir Leonard Rogers I congratulate Professor CULBERTSON on the important results of 3 years patient investigations by himself and his six colleagues at Porto Rico on the lethal action of such pentavalent antimony salts as neostibosan. At the time of my pioneer investigation on this subject in 1919 and 1920 only the far more toxic trivalent antimony salts were available. In April, 1919 I first tested the action of sodium antimonyl tartrate on *Microfilaria bancrofti* at Puri in Orissa. In four cases with large numbers in 20 cm. of evening blood I obtained great reductions in the numbers, which fell, in the one case receiving as many as eleven injections, to only 6 per cent. of the original numbers within 15 days. In order to be able to follow up cases much longer in 1919 I took advantage of infected long-term prisoners in the Cuttack Central Gaol, who could not run away and selected for treatment eight of 100 examined who showed an average of no less than seventy-three microfilariae in 20 cm. of evening blood.

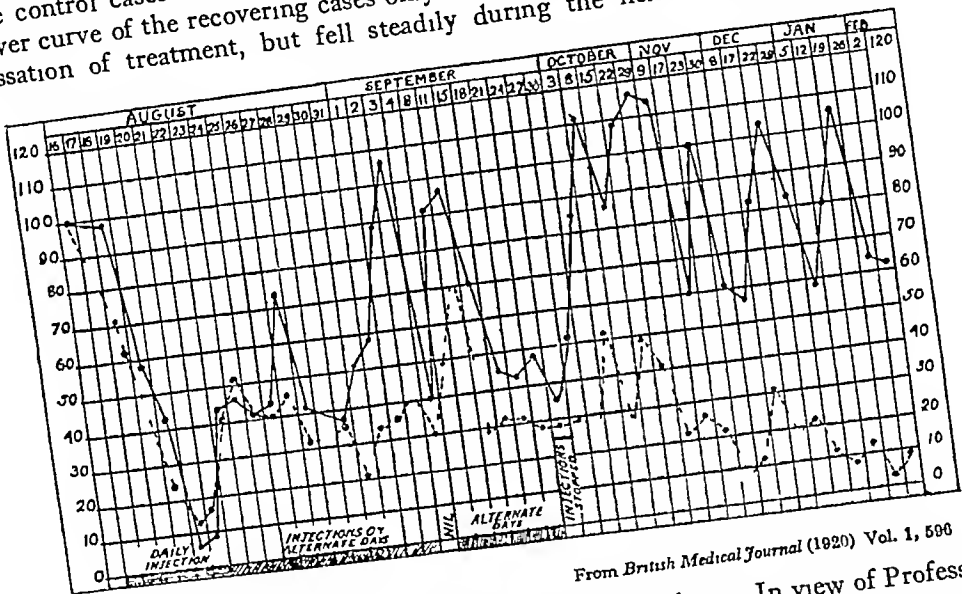
The results are clearly shown in my chart (reproduced on page 45)† Counts of the microfilaria were made by me in 20 cm. of evening blood taken at the same hour at daily and 3-day intervals during 45 days of treatment, and of 3- and 7-day intervals up to a total of 5½ months. The upper continuous line shows the percentages of the original numbers of embryos in four cases in which relapses occurred and the treatment failed the broken line shows the same data in the four cases in which it succeeded. The total dosage of 1.8 grammes was the same in each series and almost twice as much as in Professor CULBERTSON's four cases treated with tartar emetic with one success. The daily dose was rapidly pushed up to 5 c.c. of a 2 per cent. solution of sodium antimonyl tartrate, but owing to toxic symptoms from the 7th to the 45th days, with one 4-day break, 5 c.c. were given only every 6 days to only 6 and 13 per cent. of the original numbers at once rose. During the remaining period of 4½ months the numbers

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## DISCUSSION

twice rose to about the original numbers, but in the ultimately recovering cases they only once rose to over 51 per cent. It should be mentioned that counts in five control cases never fell below 45 per cent and rose to 160 per cent of their original numbers. During the last fortnight of the injections both curves showed reductions to about 45 and 30 per cent respectively, but as soon as the drug was omitted the two curves showed striking dissimilarity.

During each of the 4 months of post-treatment counts the relapsing cases all showed very sharp rises to the 100 per cent line or a little above it, the five control cases showed similar but higher peaks. On the other hand, the lower curve of the recovering cases only rose to about 50 per cent shortly after cessation of treatment, but fell steadily during the next 3 months without



From *British Medical Journal* (1920) Vol. 1, 596

treatment to only 3 to 9 per cent of the original numbers. In view of Professor CULBERTSON's proof that the embryos continue to decline after antimony treatment to the vanishing point over a period of many months, there can be no doubt that in these four cases the trivalent antimony salt had proved successful—I believe for the first time—in eliminating the *W. bancrofti* infections.

This conclusion is confirmed by the following observation, recorded in 1920 in the *British Medical Journal* of 1st May, but which has received remarkably little attention or attempts at confirmation, probably on account of the laborious nature of frequent counts over a number of months, as compared with hours in the case of studies of the diurnal variations of the microfilaria. During the peak rises, in both the control and in the relapsed cases, I invariably found very large numbers of thin young microfilariae during the counts. During the great decline in the embryos in the last 3 months of counts, in the four recovering

cases no such young forms appeared in the evening blood. I therefore concluded that the adult filarial worms had probably been killed. This is now confirmed by Professor CULBERTSON'S discovery that the adult filaria in cotton rats are killed long before the final disappearance of the embryos from the blood. In 1920 I also suggested that periodical attacks of febrile lymphangitis can be explained by lymph stasis produced by the periodical birth of such enormous numbers of microfilariae in areas where obstruction of lymphatics prevent their escape into the blood stream, but without invoking MARSON'S ingenious hypothesis of the premature delivery of ova. As early as 1904, I made numerous unpublished attempts with the aid of a centrifuge to find such aborted ova in the tissues of recently removed tumours of the scrotum without any success. In 1882, WARING in India, in an analysis of 224 cases of filarial fever found the intervals between the attacks vary from several in 1 month to one in several months. That disproves the popular Indian opinion that the attacks are influenced by lunar action—a view seriously put forward as early as 1789 by a Dr BALFOUR. WARING found the most frequent of the intervals between the attacks was in most cases either one or two a month—just those of the peaks in my control cases (not shown in the chart) and in the relapsing ones.

Confirmation, and the practical application, of my work were soon forthcoming—references up to 1929 will be found in the second edition of my *Recent Advances in Tropical Medicine*. In 1920 P. N. DAS reported on eight cases treated with twelve to thirty-seven doses of sodium antimonyl tartrate, with complete disappearance of large numbers of microfilariae in five, and great decreases in their numbers in the other three. In 1921 in Egypt, DAY also completely cleared cases of microfilariae with large total doses of the same drug. In 1920 cases of filarial fever at regular intervals, in which the attacks ceased under antimony treatment, were recorded by myself and by DAS, and a little later by BAR in the Dutch East Indies. In 1922, ROY and BOSE at Puri reported on the use of sodium antimony glycothiolate in fifty cases of elephantiasis, with diminution in the circumference of the limbs in every case, which was of a material degree in a large number of them. On the other hand, in 1924 the British Guiana Filarial Commission tested a number of drugs and found that only tartar emetic reduced the microfilariae to a limited extent. In 1929 CHOPRA and SUMNAR RAO, at the Calcutta School tried a number of antimony preparations, including neostibosan, with inconclusive results. In view of what is now reported, they must presumably have either given or followed up their cases for too short a time. The im-

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of the efficacy of antimony preparations in clearing up some *W bancrofti* infections. My research was cut short by my leaving India and retiring from the Indian Medical Service a few weeks after completing the investigation on the Cuttack prisoners in 1919-20, which I have recalled this evening. In conclusion, I suggest a re-trial of the intensive use of the trivalent antimony salts by giving three injections a day for a short time, which has recently proved successful in bringing about a rapid cure of schistosomiasis in the hands of ALVES and BLAIR, in view of the much smaller total dose of a very much less expensive drug required a point which cannot be neglected in view of vast numbers of poor persons infected with filariasis in its endemic areas. Such a trial is indicated by the nearly complete disappearance of microfilariae in my eight cases after six daily large doses of sodium antimonyl tartrate demonstrated in the chart.

**Dr L E Napier** I want to say a few words about the valuable work of Professor CULBERTSON. I think its greatest value lies in his persistence in following these cases up and in his interpretation of the results he got with the cotton rats. In the past, everybody has judged the effect of drugs in the treatment of filariasis by the number of microfilariae in the blood. One always felt that that was an unsatisfactory method as they were not the cause of the trouble, but we did not know what other method to adopt. Professor CULBERTSON has shown us that the best method was to follow up the cases for a minimum period of over a year, preferably 2 years.

At the School of Tropical Medicine in Calcutta, during the last 25 years we have used a large number of different antimony drugs in the treatment of filariasis. When I was working on kala-azar, I used to have filariasis patients sent to me for injections. The following up of the results was done by one of my colleagues, Dr SUNDAR RAO, who has done most of the filariasis work in the Calcutta School. One rather interesting result of my giving injections to these patients was that 15 years later a man met me and asked, "Don't you remember me, doctor?" I replied "No," and he said "I am the man you cured of filariasis." I was very much impressed, but my immediate reaction was to say, "Good God, the only person who has ever cured anyone of filariasis, and he didn't know it!" But I am sure we must have cured a number of our kala-azar patients of filariasis also, as neostibosan has been our standard treatment for kala-azar since 1927, 20 years ago. Saying this is not detracting from Professor CULBERTSON's work, but is rather a reflection on our own work in that we did not have the sagacity to follow up our cases and discover this fact. It is true that we never gave such large doses of a drug as he has been giving, but I think that our failure to demonstrate the value of a drug that we have used so frequently in the treatment of filariasis ever since we got the first samples from Germany in 1924, was not entirely due to this fact. I know that Dr SUNDAR RAO has followed up most of his cases, examining



their blood at intervals, and he has often expressed the belief that he was getting good results with this drug but he was never quite satisfied, and I think the reason is that he did not follow them long enough. It is of course true that he also was working in an endemic area where there was danger of re-infection.

**Dr F Hawking** I should like to pay my tribute to the work of Dr CULBERTSON and his colleagues which, together with the similar work done by BROWNE\* constitutes the first real advance in the chemotherapy of human filariasis. In 1938 I had an opportunity in East Africa of treating filariasis patients with antimonial and other compounds † Particular attention was paid to fuadin, which was pushed to the toxic limit. But at the end of the course, microfilariae were still present in the peripheral blood and it was concluded that no therapeutic action had been exerted, since it had not been realized that an active drug might kill the adult worms while leaving the microfilariae apparently intact. It is Dr CULBERTSON's great contribution to have shown that patients should be followed up for 6 months or more to detect this long delayed fall in the microfilariae count which he has described to us. However the work done in East Africa was enough to show that man is a very unsatisfactory test animal for preliminary work in chemotherapy and that for more rapid progress it was necessary to find a suitable laboratory experimental animal which should be small, cheap and easy to handle. Work in America between 1943 and 1945 indicated that the filariasis of cotton rats might provide the laboratory infection that was desired. CULBERTSON and ROSS showed that wild cotton rats with spontaneous infections of *Leishmanoides* were convenient for experimentation. And WILLIAMS and J. A. SCOTT showed that this infection was transmitted from one rat to another by the tropical rat mite, *Liponyssus bacoti*. At the end of 1945 I was privileged to visit the laboratories of all these workers, who very kindly showed their methods and results. They also provided infected rats and strains of *Liponyssus* which were brought back to London and established at the National Institute for Medical Research. As I knew that Professor GORDON, in Liverpool, was interested in cotton rat filariasis, I gave him the information, as early as possible, that the vector he was seeking was *Liponyssus*.

At Hampstead, we have concentrated on the large-scale transmission of the infection so as to obtain big numbers of infected rats for chemotherapy. With reasonable care and experience it seems quite possible to produce any desired number of infected rats in the laboratory in this country. At the beginning we tried to see whether other animals besides cotton rats could not be infected. The worms can be passed into ordinary laboratory rats, mice, hamsters or even Orkney voles, and they can develop to maturity and produce microfilariae. But the worms do not seem nearly so happy in these animals

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† HAWKING, F. (1940). *J. trop. Med.* 43 204.

as they are in cotton rats—the infections are light and they tend to die out spontaneously. Accordingly, cotton rats are the only hosts found really suitable for this work. We have repeated the work of Dr CULBERTSON and his colleagues with antimonials and our experience has been similar to that which he has described. We also found that in cotton rats arsenical compounds, such as tryparsamide and its trivalent derivative, are as active, or even more active, than antimonials—a fact which had already been recorded by BRADY and by OTTO in America. Dr CULBERTSON has described the therapeutic effect of one arsenical compound—melarsen oxide—and this particular arsenical seems less suitable for clinical use than neostibosan. But melarsen oxide is a somewhat atypical compound, and a thorough trial should be given to other better known arsenicals, *e g*, tryparsamide, neoarsphenamine, and mapharsen, before arsenicals are abandoned.

Besides antimonials and arsenicals, a long series of compounds has been investigated, including acriflavine, sulphonamides, marfanil, methylene blue, neosolganal, pamaquin, phenanthridinium 1553, etc, and none of these was active. However, some therapeutic effect has been found in two other types of organic compound, since work on this subject is being pursued intensively in America and at Hampstead, it is quite likely that compounds will be discovered in the next few years which will be very active in the treatment of this disease. The ideal remedy sought is a compound which is very active, non-toxic, cheap, and capable of being given by mouth.

Finally, I should like to offer an observation on some of Dr CULBERTSON's results. Although most of his patients responded well to neostibosan, there were a few in which the microfilariae persisted obstinately in the blood stream. In our cotton rats, treated with antimonials, we found, as Dr CULBERTSON has described, that the adult worms were killed by the drug while the microfilariae persisted and remained actively motile. But we found in addition that half-grown immature worms were also much more resistant to the drug than were the adult worms. A similar phenomenon has been seen in Germany by KIKUTH and GONNERT in the treatment of experimental infections of *Schistosoma mansoni* by certain new organic compounds. Here, also, immature worms have been found to be completely resistant to doses which easily kill the adult worms. It is possible that in those cases of Dr CULBERTSON's which did not fully respond to the treatment, immature worms were present due to recently acquired super-infection. These immature worms may have resisted the treatment and gradually replaced the earlier generation of microfilariae by a new crop. If this is the case, it may form a definite handicap to the chemotherapeutic treatment of filariasis.

**Professor N Hamilton Fairley** I would like to congratulate Dr CULBERTSON on this very admirable paper. Reference has been made to the experimental approach to the subject, but from a clinical point of view what

is equally important is the stress laid by Dr CULBERTSON on his control series of cases. It seemed possible that re-infection had occurred in one of these patients and I would like to ask Dr CULBERTSON what was the probability of re-infection in other cases. If re-infection was in fact occurring, then the results were even better than Dr CULBERTSON's data indicated.

In regard to *Loa loa* infestation, much remained to be learnt regarding the natural course of this disease in untreated patients. It appeared that neostibosan acted on adult filaria worms just as trivalent antimony compounds did in schistosomiasis. Further investigation on the complement fixation reaction in *Loa loa* infestations as an index to what was happening to the adult worms would be interesting and possibly of real value.

Sir Philip Manson-Bahr I do not think I can contribute much to this discussion except to thank Dr CULBERTSON for his admirable contribution tonight. We have admired his presentation, his clear diction and his humour—which is always very useful. Perhaps I might say one or two things from a clinical point of view as the result of the treatment of filariasis in people returning from the tropics, whom I have been able to watch over during a quarter of a century in London. Dr CULBERTSON wanted data of the manifestation of loiasis on people returning from West Africa. I have seen a large number of these people, and one thing has struck me, that Calabar swellings are often much more troublesome and apparent a few months after arrival in a cold climate than they were in West Africa. I have had patients objecting to coming home on leave because of the inconvenience of these swellings, which may persist for many years after the return from the tropics. I had one man who went on having Calabar swellings for 17 years until the microfilariae disappeared, but in others they soon cease to be troublesome. One lady suffered badly for 7½ years until the disease ended in the most dramatic way. She got attacks of fever and then subcutaneous abscesses in various parts of her body. When they were opened there appeared in the pus the calcified remains of *Loa loa*. We see from these clinical observations that *Loa loa* does not disappear spontaneously on removal to a colder climate. As regards *Wuchereria bancrofti*, the clinical manifestations as one sees them in the tropics—the attacks of lymphangitis—tend to disappear much more quickly—in 3 or 4 years—except chyluria, which may persist throughout the life of the patient. As regards *Onchocerca volvulus* infection I have had only a few cases, but two were serious ophthalmic cases from Kenya. Both became blind. Biopsies of the skin were done at definite periods during 2½ years, during which time in this country the microfilariae disappeared without specific treatment. But that did not arrest the progress of the disease which went on to complete blindness. By a very skilful operation one man has had his cornea removed and a new one grafted on and that has restored the sight of one eye. Treating *Onchocerca volvulus* with neostibosan seemed to make the eyes worse, and I stopped it. I would like to

congratulate Dr CULBERTSON on his very admirable control experiments because, especially with *W bancrofti*, when whilst observing patients for many days, weeks and months, and making regular microfilariae counts, one is struck with the disappearance in the number to be found in the blood from time to time. Every now and again they mysteriously disappear without any drug treatment. The other point is the interpretation placed on the remains of dead filariae in the scrotum and other parts of the body. Such observations as I was able to make many years ago—confirmed by Dr F W O'CONNOR in his well-known work—show that filariae may die of old age or injury, and are sometimes found in the centre of a blood clot without taking any drug whatever, and one must be careful not to interpret results as necessarily due to a drug given in any particular instance.

**Mr L G Goodwin** I was very much interested in the antimony figures for blood. This approach should help us to find out what is happening in these cases. The highest blood levels were for the drugs that worked, and I think the low levels for the trivalent compounds were the result of toxicity limiting the size of dose given. In the case of stibanose the low level was probably due to a rather more rapid excretion of the drug as compared with neostam and neostibosan. It would be interesting to find out what was the valency of the drug in the circulating blood. Application of the polarographic method used by Dr PAGE and myself would show whether or not the antimony in the blood is in the trivalent state. When pentavalent antimony is injected, the part that is not rapidly excreted remains in the liver and is gradually released into the blood stream from there. A great deal of the antimony in the liver is trivalent, and I think it may be possible that intensive drug treatment gives rise to a liberation into the blood of trivalent antimony over a prolonged period, and that may be doing the job.

**Dr R D C Johnstone** I may be excused for making one or two personal observations because I am now suffering from a *Loa loa* infection. First of all, in respect of what Sir PHILIP MANSON-BAHR has just said, the disease alters very considerably when one gets back to a temperate climate—or at least it has done so in my case. I am not under treatment and I am getting very few Calabar swellings now. Can Professor CULBERTSON give us the reason for the toxic changes which took place in patients with *Onchocerca volvulus*? I feel that any assessment of treatment will be most difficult because, whether we are in an endemic area or a temperate climate, the disease fluctuates from time to time, and the interpretation of results in these loiasis cases must be treated with extreme caution.

**The President** There is one question I should like to ask Professor CULBERTSON. He has told us that under treatment with neostibosan there was

evidence that the adult filaræ died, and that the embryos remaining in the blood disappeared gradually during the course of a year or longer. I should like to know whether during this time the embryos showed the periodicity they had shown before? It may be that Dr CULBERTSON and his colleagues formed the habit of examining the blood only at night, in which case it would be impossible to say whether there was any periodicity or not.

Professor Culbertson (in reply) I am asked by Dr JOHNSON to explain the toxicity seen in the *Onchocerca* cases, but I am at a loss to do so. It troubled us. Up to the time we had difficulty in the onchocerciasis cases we had thought that we could use neostibosan with almost an assurance that there would be no trouble. I have no explanation why the Mexican onchocerciasis patients seemed to suffer from it whereas the cases of bancroftian filariasis in Puerto Rico and the few subjects treated in New York, did not manifest evidence of toxicity. With regard to the periodicity of the filaræ after treatment as Dr WENTON suggested, it was our intention and effort to examine the same patient at the same hour each evening and very few were examined at other times. I am afraid I have no more data. I have no information about the valence of the antimony in the blood, although I am glad to know that the polarographic method will tell what the valence is. I am not a chemist and was not personally engaged in that part of our work. All the antimony determinations were made by Dr GILLHORN of the Department of Pharmacology in our institution. I am glad to learn from Sir PHILIP MANTON-BAHR that the Calabar swellings may continue to appear over so many years. I realize that the nodules we saw in the scrotal sac of certain bancroftian cases may represent old worms killed by other means. On the other hand, Dr KOPFISCH the pathologist at the Puerto Rico School of Tropical Medicine, in examining the sections, said that the excised worm was recently dead because of the condition of the ovarian nuclei, and we felt justified in believing that the worms in these other nodules had probably been killed as the result of our specific treatment. I see no reason,

however, why worms dead from any other cause might not be found in the individual. If you let infected cotton rats rest for a year or two, then kill them, you may find in the pleural space masses of filarial worms resembling in many respects those that I showed in one of the first slides. The same typical foreign body reaction to these dead worms will develop no matter whether the worms are killed by neostibosan, or any other drug, or whether they die of old age or injury. As to Professor FAIRLEY's statement many of our patients, including the one in which I suggested there might have been re-infection, were inmates of a children's home, and although they slept under mosquito nets at night, with individual nets over each bed, nevertheless there were excellent opportunities at other times for the children to become re-infected. There were many mosquitoes about, these often of the *Culex* variety, and I see no reason why there should not have been adequate opportunity for the re-infection of some patients. We could not regulate such things.

I think the point that Dr HAWKING suggests is very logical. He agrees that the microfilariae are more resistant than the adult worms to the drugs, and it seems to me very logical that an immature worm, beyond the embryo stage, might also be more resistant to the effects of the drug than adult parasites. I believe that male worms in general are considered to be more resistant than the females to various kinds of drugs, and I think I can say from memory that where occasionally there was a single survivor in the pleural space of one of our treated cotton rats, that single survivor was usually a male. There might be a surviving female or two but the chances were better that survivors were males.

In reply to Dr NAPIER—but this is not exactly a reply—we adopted the intensive schedule of neostibosan treatment only after consulting with him in New York and we are very grateful to him for his suggestions. He was in New York, came to us at our request and talked over our work. On our earlier first visit to Puerto Rico we had been exceedingly cautious about dosage. We gave a single 300 mg dose per day and often omitted drug for a few days thereafter to rest the patient. After talking with Dr NAPIER, who years earlier had given larger doses than we had given, and had gotten away with it, we felt justified in being more venturesome.

Some of you may know that I came to England last January to look into the life and work of PATRICK MANSON, although the task has been most pleasing, thanks to Sir PHILIP MANSON-BAHR and others whom I have interviewed, the job of finding something new on my subject has not been an easy one. Sir PHILIP, in his biography of 1927, performed a notably complete work, so that all that remains is for me to try to do the same thing but differently. In fact, there is only one item that I consider significant, and which seems to have escaped Sir PHILIP appropriately for this evening this concerns an experiment by

MANSON in chemotherapy on a case of bancroftian filariasis. I should like to close my discussion by presenting MANSON's data found on pp. 525-526 of his *Amoy Diary* which I invite you to interpret in the light of my own data already given this evening.

1892	Nov 26th.	placed on thymol (tablets) 4 grains four times daily and to keep this up for 2 months.
	Dec. 6th.	In good health—taken 16 grains thymol daily. A rather small slide drawn about 9 p.m. contained 120 filariae.
	" 18th.	Plenty of filariae in blood.
	29th.	A full slide drawn about 8.30 contained 359 filariae. Patient in good health.
1893	Jan. 16th.	Two slides drawn about 10 p.m. contained 327 and 309 filariae respectively. No ill effect whatever from the thymol which has been taken repeatedly since commencement of experiment.
	Nov. 6th.	10.30 p.m., 1 filaria.
	10th.	10.20 p.m., 1
	" 10.30 p.m., 6	
	" 12th.	10.30 p.m., 1
	14th.	10.30 p.m., 1
	" 16th.	10.20 p.m., 6

I think I can see enough similarity between these observations and my own to let me suspect that the treatment MANSON applied may have been at least a contributing cause to the apparent cure and I may be correct in saying, then, the earliest recorded data on the successful chemotherapy of bancroftian filariasis can be found in MANSON's *Diary*. It is a pleasure for me to acknowledge MANSON's priority.

## ORDINARY MEETING

of the Society held at  
Manson House, 26, Portland Place,  
on

Thursday, 15th May, 1947, at 8 p m

THE PRESIDENT,  
C M WENYON, C M G, C B E, M B, B S C, F R S,  
in the Chair

### DISCUSSION

on

### AMOEBIASIS

Dr C M Wenyon (in opening the Discussion) Interest in the subject of amoebiasis seems in some way to be stimulated by great wars. This was the case during and after the 1914-18 war, and it appears that again the same thing has happened in the war that has recently ended. The reason for this seems clear and applies not only to amoebiasis but to many other diseases. It is due to the despatch overseas to areas where these diseases are endemic of very large numbers of susceptible individuals, many of whom quickly fall victims to the infections to which they become exposed. In the case of amoebiasis, and other infections also, it is probable that the percentage of those that become infected is actually not greater than that which occurs in times of peace amongst those whose duties take them to the same countries, but the actual numbers are greater and in consequence there are more of the chronic cases which are refractory to emetine or other treatment. One would like to know what happens to these chronic cases. Do they all succumb to their infections sooner or later or do they gradually recover? Just after the 1914-18 war there were many of these cases in hospitals which did not appear to be relieved by any kind of treatment. Where are they now? And similarly after the recent war, one hears of many of these cases which fail to respond to any treatment.



It is very largely this failure which has suggested our discussion on amoebiasis this evening, and I hope that as a result some light may be thrown on some of the as yet unsolved problems connected with it.

It is well known to many of us that at the beginning of the 1914-18 war the despatch of troops to Gallipoli and Egypt was followed by an epidemic outbreak of diarrhoea and dysentery and the conclusion was drawn, mainly because of lack of experience of the medical officers who had to deal with the epidemic, that the condition was primarily amoebic dysentery. All kinds of cells were wrongly identified as amoebae and little thought was given to the possibility that the main disease was bacillary and not amoebic dysentery. When better diagnostic facilities became available it was realized that the majority of cases were bacillary dysentery and that amoebic dysentery was responsible for at most, only 10 per cent. of the cases. In the war that has just ended this great mistake was not repeated, though the advent of sulpha-guanidine may have prevented so much bacillary dysentery that the percentage of amoebic cases may have been relatively higher than 10 per cent. of the total.

The supposed epidemic of amoebic dysentery associated with the Gallipoli campaign raises the whole question. Do epidemic outbreaks of amoebic dysentery occur? CRAIG (1944) and others who follow him hold that they do, and in support of their contention they quote the now famous outbreak which occurred in Chicago in 1933-34 and was said to be largely due to a local polluted water supply in two hotels. In all there were said to have been over 1 400 cases and over 100 deaths. I doubt very much whether it would be possible for drinking water in a hotel to be contaminated with the huge doses of cysts which would be necessary to give rise to acute amoebic dysentery in 2 days as was supposed to be the case in some instances. Would the hotel guests not have seen that the water was impure and refused to drink it? The report on the outbreak admits that, experimentally extremely large doses of *Entamoeba histolytica* have to be administered to both man and animals to produce infections comparable to those observed in the epidemic in which the incubation period was short, the lesions very severe and the exposures frequently minimal. It is my belief that the outbreak was mainly a bacillary one in a group of people in which the *E. histolytica* carrier rate was high, as it admittedly was and is in Chicago and many other cities in the U.S.A. I expressed these views in an unsigned article which appeared in 1934. I recently came across a paper on "Complications of Amoebiasis," by SIMMONDS (1943) in the U.S.A., in which reference is made to the Chicago incident. It is evident that SIMMONDS has grave doubts as to the epidemic character of the outbreak, for he writes —

It is likely however that few physicians who would constantly bear in mind the prevalence of this disease and few laboratory technicians capable of finding and identifying *E. histolytica* could start an epidemic of amoebiasis in any city in this country in the same way that Lincoln Steffens started crime wars in New York City by merely revealing pre-existing but unreported and unsuspected cases.

The life history of *E. histolytica* is now well known. It has been studied in man and animals and has been successfully cultivated, yet there are many points which are still not clear. How is it that there are a far greater number of symptomless carriers who pass cysts than there are cases of amoebic dysentery, and why is it that *E. histolytica* will live and feed on the tissues or fluids in the wall of the intestine or in liver abscesses in the absence of bacteria, yet it will not grow in culture unless bacteria on which it can feed are present? The question of the carriers is one of peculiar difficulty. It first attracted attention during the 1914-18 war, though WALKER and SELLARDS in 1913 in Manila had administered cysts from healthy carriers—some of whom had never suffered from dysentery—to sixteen volunteers. Of these, some fifteen became infected with *E. histolytica*, and four subsequently developed amoebic dysentery. The outbreaks of dysentery in Gallipoli and Egypt to which I have already referred led to the examination not only of those who actually had dysentery and those who had recovered, but finally of those who had never been in areas where amoebic dysentery was common. These examinations showed that amongst apparently healthy individuals a varying percentage were carriers. Thus, amongst over a thousand new recruits examined by MATTHEWS and MALINS SMITH in Liverpool during the 1914-18 war as many as 56 per cent had cysts of *E. histolytica* in the stools. Such examinations have been continued by observers in all parts of the world and it can safely be stated that *E. histolytica* infection is widespread amongst apparently healthy populations everywhere. The extent of the infection varies in different localities but it may be as high as 53 per cent, as in a group of 154 students—many of whom admittedly had recently returned from service overseas—examined by KOFOID and SWEZY at the University of California. A common figure is 10 to 20 per cent, but often it is considerably lower than this. Though the actual figure recorded depends largely on the methods of examination, the number of times each individual is examined and the patience and ability of the person making the examinations, there is throughout the world a great reservoir of *E. histolytica* infection in people who appear to be quite healthy. This being so, it is strange that there is so little correlation between the incidence of these healthy persons and the cases of amoebic dysentery. In the tropics cases of amoebic dysentery are commoner than in colder countries and it is well known that healthy persons such as, for instance, soldiers going overseas, amongst whom carriers of *E. histolytica* occur, may develop amoebic dysentery soon after they arrive in the tropics, when it is safe to assume they—and I am thinking particularly of the healthy carriers—would not have done so if they had stayed at home. When they get amoebic dysentery, is this due to the strain of amoeba they were carrying when they went out, or is it due to some newly acquired strain? If it is due to the original strain, then one must suppose that there has been some change in the intestine which has enabled the amoeba already there to become pathogenic. Such a change might be brought about by the many bacterial

infections which give rise to mild and serious intestinal upsets which are so common amongst new arrivals in the tropics. Shortly before the war which has recently ended, WESTPHAL claimed to have shown by experiments on himself and others that the administration of bacilli, which had been cultivated from a case of amoebic dysentery to healthy carriers of *E. histolytica* led to the development of amoebic dysentery which followed upon the acute bacterial infection. On the other hand there are those such as BRAUPT who have advanced the view that the amoeba of the carrier in temperate climates, though morphologically indistinguishable, is quite distinct from that causing amoebic dysentery. This hardly seems to be a correct explanation, for there is ample evidence that in kittens the amoeba from the healthy carrier in temperate climates may give rise to as acute an infection as do the amoebae from acute cases of amoebic dysentery in the tropics.

A very difficult question to settle is that of the condition of the intestinal mucosa in the symptomless carrier of *E. histolytica*. A number of observers have noted that individuals who have died of causes other than amoebiasis and who have had no history of dysentery may reveal postmortem quite extensive amoebic involvement of the large intestine. Thus MUMFORD (1910) in the Philippines reported on the occurrence of typical amoebic ulceration in fifty cases which had died of diseases other than amoebiasis and in which there was no history of diarrhoea or dysentery. There seems often to be little parallel between the degree of involvement and the actual symptoms noted. It is evident, therefore, that amoebic ulceration of the large intestine of varying intensity may occur in individuals in whom amoebic infection is quite unsuspected. On the other hand, are there cases of known amoebic infection in which no ulceration whatever is present—in other words are there really perfectly healthy carriers? In an attempt to decide this issue FATER (1941) examined the intestine of 202 individuals who had died of accidents in New Orleans and showed that thirteen harboured *E. histolytica* in the large intestine. Of these, seven revealed superficial lesions of varying extent which could be attributed to the amoeba. In six, no lesions could be detected, in spite of the fact that in four of them amoebae occurred throughout the whole length of the large intestine. In two infection was revealed by the discovery of a single cyst in each case. Thus in these cases it may be said that there may be a quite extensive amoebic infection without any lesions whatever being detected. Some evidence has been obtained from the examination of monkeys which, as everyone knows, are liable to the same intestinal protozoal infections as human beings. Thus JOHNSON in Panama kept under observation seven indigenous spider monkeys and four house monkeys from India with *E. histolytica* infections. One of the spider monkeys suffered from diarrhoea developing into dysentery. It was observed till its death in 98 days. Amoebae, but no cysts were constantly present in the stool, and after death it was found that the mucosa was almost completely destroyed and that the sub-mucosa was extensively invaded by amoebae. The remaining ten monkeys were kept under observation for 40 to 623 days before being

sacrificed for postmortem examination. During observation cysts were constantly passed but there were no symptoms associated with the infection. At postmortem examination the large intestine showed no macroscopic lesions in nine, while one showed a granulation of the caecal mucosa. When sections were examined microscopically, lesions containing *E. histolytica* were found in seven while in three no lesions were detected.

More recently, BOND, in America, has made a minute examination of the intestine of five rhesus monkeys which were symptomless carriers of *E. histolytica*. In all of these the large intestine revealed no gross ulceration, but microscopic examination again revealed in all the animals a patchy low-grade chronic inflammatory reaction with interstitial haemorrhages and, rarely, shallow ulcers. Amoebae lay in the gland crypts and some had invaded the mucosa. In this case, though there was some tendency for the amoebae to invade the mucosa, the invasiveness was of a low order (BOND *et al* 1946).

FAUST and his co-workers have recently reported upon a study of eighteen strains of *E. histolytica* obtained in culture from a variety of different sources, including carriers and actual dysenteric ulcers. They have found not only that the strains vary in their cultivability but that there is considerable variation in their infectiveness to kittens, as also in the extent to which lesions are produced. In some cases it appeared that infection had occurred without the production of any visible lesion. They conclude that cultivability, infectiveness and power of invasion are three qualities which do not necessarily run parallel with one another (FAUST *et al* 1946).

From these observations on human beings and monkeys it is clear that the extent of invasion of the intestinal mucosa varies considerably and that sometimes, as in the monkeys, when there is no visible macroscopic lesion, examination of sections microscopically will reveal that some invasion has occurred. In monkeys the intestine can be examined immediately after death before any postmortem change has taken place, but with human beings this is never possible. In the case of the postmortem examinations made by FAUST in New Orleans, referred to above, these were all carried out within 4 hours of death by accident, yet in four of the cases, though amoebae were found throughout the large intestine no lesions could be detected. It is possible, however, that if extensive examination by microscopic sections had been carried out, as in the case of the monkeys studied by JOHNSON and BOND, invasion might have been revealed. I remember that some years ago I discovered a small amoebic ulcer when examining sections of the large intestine of a man who had died of some condition other than amoebiasis. There had been no indication of dysentery and amoebic infection had not been suspected. The ulcer, which was limited to the mucosa, had a diameter of about 1 mm and the amoebae in it were particularly well preserved. A section of this ulcer served for an illustration in my "*Protozoology*". The case was probably one of a carrier, but whether there had been a history of previous dysentery I cannot say. If I were asked "Does *E. histolytica* ever live in the large intestine of man

without giving rise to lesions, however minute?" I should have to answer "I do not know." We know it can live in cultures feeding on bacteria, but whether it can survive in this fashion in the lumen of the intestine without abstracting nourishment from the tissues no one can say. Perhaps it lives on the surface of the mucosa in the layer of mucus which covers it, and actual invasion only occurs when other agents such as bacteria intervene.

The other day I came across a slide I had prepared over 20 years ago. It was labelled "*E. histolytica* cysts self." What kind of infection this represented it is hard to say. It is perhaps safe to assume that I was a carrier, but it is impossible to tell whether the trawings from which I suffered from time to time as all of us do were or were not due to my particular brand of *E. histolytica*. However that may be without any treatment the infection has long since disappeared, though the trawings are no less or no more frequent than they were during my infection.

A question that is often put to me is how often should a case be examined in order to exclude an amoebic infection. The answer I should give would be "as often as possible." In this connection I might recall that in Egypt during the 1914-18 war the late Dr F. W. O'CONNOR and I attempted to answer this question by examining a series of healthy soldiers about a dozen times. In all, ninety-two persons were examined, about 10 minutes being spent on each specimen. Of the ninety-two examined, twelve were found to harbour *E. histolytica*. The general result was that at the first examination only four were found infected, whereas as a result of all the examinations this figure was trebled. Some cases were first found to be infected at the third examination, others at the sixth, while one was first detected at the tenth examination. It is possible that with further examinations other infections would have come to light. In practice when I am asked to exclude an infection, I generally ask for a specimen once a week for 8 weeks—the specimen being an ordinary normal one not procured by a saline purge. I have no experience of detecting infections by culture methods but I doubt whether these would disclose many infections which could not be detected by a series of careful microscopical examinations. Again, I very much doubt whether the complement fixation test as advocated by CHAIN will help very much in detecting infections which cannot be otherwise determined. Perhaps it is that, being a protozoologist, I always prefer to see the organism itself rather than rely on some conjectural test which at times may lead me astray.

Another question of some practical importance is the presence of red blood corpuscles in an amoeba. If amoebae are seen with included red blood corpuscles, are we justified in stating that these are *E. histolytica*? It is true that in cultures *E. coli* may be induced to ingest red blood corpuscles, while TRYZZER and GRIMAN have described a case of undoubted *E. coli* infection in which on a number of occasions the amoebae included red blood corpuscles. It has to be admitted, therefore, that very exceptionally an error would be

committed by concluding without other evidence that any amoeba with included red blood corpuscles must be *E. histolytica*. But the presence of red blood corpuscles in *E. coli* occurs so very rarely that for all practical purposes this possibility can be ignored. I know of no case in which I have seen amoebae with included red blood corpuscles which I have had reason to believe were *E. coli* rather than *E. histolytica*.

A point of considerable interest is the significance of the small race of *E. histolytica*. In Egypt during the 1914-18 war O'CONNOR and I noticed that certain cases passed regularly cysts which were very much smaller than the usual forms. It appeared to us that these were races which differed from one another in the size of the cysts. We could not satisfy ourselves that these small races were associated with dysentery. Since then they have been studied by a number of observers and it is generally agreed that two races occur—the one producing cysts of the ordinary type, over  $10\mu$  in diameter, and the other smaller cysts under  $10\mu$ . One of these small races was cultivated by FRYE and MELENEY (1938) through many sub-cultures for a period of 8 months, during which the cysts and amoebae retained their small size. With the culture amoebae twenty-two kittens were inoculated and five became infected. The lesions in these kittens were superficial, there being little tendency for the amoebae to penetrate the tissues as do the amoebae of the large race. It is of interest to note that the case from which the small race was cultivated gave a positive complement fixation test which became negative after treatment. The question of the behaviour of the small race in the human intestine is difficult to decide. There do not appear to have been any satisfactory records of frank amoebic dysentery in man caused by the small race of *E. histolytica*. We must suppose that the tendency to damage the intestinal wall is much less in the case of the small race than in the large, so that actual dysentery rarely if ever occurs.

In concluding these remarks, I should like to say a few words about the treatment of amoebic infections. There are two classes of case which present themselves for treatment. The healthy, or symptomless, carrier and the patient with frank amoebic dysentery. The healthy carrier is one who is usually discovered when microscopical examinations are made for purposes of surveys of intestinal protozoal infections or during routine examinations of patients in hospitals. They are discovered accidentally as it were, but the discovery immediately raises the question of treatment. Should attempts be made to get rid of the infection, or should nothing be done about it? In my own case there was no treatment and the infection disappeared during the course of a few years. On the other hand, it is well known that cases of fatal amoebic dysentery and fatal amoebic liver abscess do occur from time to time in this country in people who have never left it. It would seem, therefore, that having discovered an infection the physician has the responsibility of trying to get rid of it. As a matter of fact, when such apparently healthy carriers are closely questioned it is often possible to obtain a history which would seem

to indicate that the infection is not as innocuous as at first sight it might appear to be. The treatment of the isolated carrier who comes to light does not mean that it is advisable to institute wholesale examinations with a view to the detection and treatment of every carrier. This would be quite outside the realm of practical politics as in every country there are so many of them about. Cases of acute amoebic dysentery of course demand immediate attention, for which emetine in one form or another is the specific remedy—specific in that it usually cuts short the attack and relieves the symptoms, but non-specific in that too often it fails to eradicate the amoebic infection entirely so that relapses occur. Between the so-called healthy carrier condition and that of acute dysentery there is a whole series of cases in which varying degrees of looseness of the bowel, alternating with periods of constipation and diarrhoea with vague abdominal pains are the characteristic features. Mucus and blood and intestinal cells are often present, while the amoebae are sometimes the large tissue-invading forms, some of which may show included red blood corpuscles and at other times the small pre-cystic amoebae with their cysts. Treatment of these cases is again by emetine, but, as in the acute cases, relapses are not infrequent. Some cases fail to respond to treatment at all and are completely resistant. You will remember that HARGREAVES (1946) suggested that in some cases emetine failed to act because of concomitant bacterial infections and he was able to show that following penicillin and sulphonamide treatment which, presumably eradicated the bacterial infections the emetine treatment was successful. How are we to suppose that the bacteria interfered with the action of emetine? Is it that the bacteria produce such changes in the intestinal wall that emetine cannot get at the amoebae? Even this procedure fails in certain cases in which a condition of chronic colitis exists. I have sometimes wondered whether in some of these very refractory cases there may not be a mixed condition of amoebic infection and ulcerative colitis. It has been suggested that emetine resistance may be acquired by strains of *E. histolytica* but there has been no satisfactory evidence that such emetine-resistant strains exist.

I have spoken of emetine treatment without any reference to the quantity or form given. The present tendency is to administer it in the form of emetine bismuth iodide, three grains a day for a number of days. With this it is the custom to give other drugs such as diodoquin and carbarsone in various combinations. Recently the following procedure was advocated by PAOR (1946). Treatment extends over 20 days. Each day 3 tablets of diodoquin are given three times a day throughout the 20 days. In addition, on the 1st and 2nd days 1 grain of emetine is given intramuscularly. On the 3rd to the 14th days (that is for 12 days) 3 grains of emetine bismuth iodide are given each night and to finish up on the 15th to the 20th days (that is 6 days), 4 grains of carbarsone are given each day. The number of treatments of the kind is very great and most physicians have their own pet combinations, but it rests with those who are more experienced than I am to say which is best.

There is, however, one point to which I should like to call attention in this connection. It is that in Egypt during the 1914-18 war O'CONNOR and I tested a number of different methods of giving emetine for amoebic infections and we found that the best results were obtained by giving each day for 12 days a grain injection subcutaneously each morning and half a grain in capsule each evening by the mouth. It may be that this dosage is too high, but we found that with it we had the greatest number of permanent cures.

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Dr F Murgatroyd. For the clinician, the main problems of amoebiasis concern diagnosis, treatment, and assessment of cure, the first and the last being essentially the same. My remarks will be concerned chiefly with intestinal amoebiasis.



## DIAGNOSIS.

As amoebiasis implies infection with *E. histolytica* diagnosis depends on direct or indirect demonstration of this amoeba, despite any influence the bacterial flora or chemical composition of the faeces or any other factors, may have in determining the infection with the amoeba.

## DIRECT MICROSCOPY

In intestinal amoebiasis, direct microscopic examination of freshly passed stools remains the basic diagnostic procedure. Although, as WINTER and O'CONNOR (1917) wrote, if *E. histolytica* is going to give trouble, the probability is that the amoeba or its cysts will be found at once, difficulties arise when the parasites are scanty as in certain chronic infections and as in tests of cure after treatment.

*Saline purgatives*.—Administration of a saline purge may increase the chance of finding amoebae ANDERSON (1934) stating that almost 90 per cent. of infections were discovered by a single stool examination after a purgative whereas without purgation six daily examinations of the same individuals detected only 75 per cent. of the infections on the other hand there are some who deny that purgation makes any material difference. Cultural methods may help, but sometimes cultures are negative from stools known to contain parasites. Concentration method may double the chance of finding cysts in a given specimen but the methods are not suitable for vegetative amoebae.

In negative cases, examination of six or more consecutive stools is commonly recommended, and for test of cure such a series of examination may have to be repeated at intervals for several months. An important question is, therefore, how many negative examinations are required before it is reasonable to assume that a patient is not infected.

## PROVOCATIVE EMETINE.

Recently provocative emetine has been recommended as an aid in diagnosis, the suggestion being that after a dose of emetine amoebae are found more readily in the stool. Neither in the published papers nor in response to enquiries from certain of the authors however does there appear satisfactory statistical evidence that this occurs. Furthermore if a dose of emetine does increase the number of parasites appearing in the stool it would seem that it could only do so by expelling those already in the gut or by stimulating their multiplication. One might expect therefore that either a change in the character of the motion would occur after a single dose of emetine, or that small amounts of the drug would stimulate division of amoebae *in vitro*. So far I have not been able to confirm that these phenomena occur. It seems that there may be some confusion with the use of

"provocative arsenic" in the diagnosis of syphilis. In this case, however, diagnosis does not depend on an increase in the number of parasites but upon the development of a positive serological reaction following their destruction and the liberation of antigen with consequent stimulation of antibody. The mechanism differs therefore diametrically from that presumably concerned in "provocative emetine," the use of which would appear as reasonable as administering an anti-malarial drug to increase the chance of finding malarial parasites in a subsequent blood film. If, however, "provocative emetine" is of value it is important that the fact be established and I would suggest, therefore, that those who have so found it should publish the data upon which their findings rest.

#### SIGMOIDOSCOPY

The value of sigmoidoscopy and proctoscopy has also been stressed, one advocate stating that many patients "come to light as cases of amoebic dysentery solely as the result of sigmoidoscopy." While such procedures may help in assessing the degree of disease and may occasionally reveal some unsuspected condition such as a carcinoma, their value in the positive diagnosis of amoebiasis in my experience is slight. Firstly so-called typical amoebic ulceration is usually reflected in positive stool findings, while in the difficult cases the appearances of the bowel alone are insufficient and may be misleading, small localized ulcers in an otherwise normal looking mucous membrane may be associated with bacillary infection while diffuse granular conditions may be amoebic. Secondly, the lesions must be within reach of the instruments, namely in the rectum or sigmoid. Although the writer I have just quoted states that "the maximal incidence of the commonest lesion of amoebic dysentery is 3 to 6 in from the anus," ROGERS (1922) and CLARK (1925) found from autopsies that only 66 to 75 per cent of infected patients have lesions in the rectum or sigmoid, and CRAIG (1944) suggests that if this is the position in fatal cases then probably the incidence in mild or latent cases is much less and may be only 25 per cent.

#### X-RAY EXAMINATION

Much less valuable in the diagnosis of intestinal amoebiasis is X-ray examination. Although a recent paper states that "the amoebic caecum (by barium enema) gives a distinctive appearance," I am inclined to agree with the conclusion expressed by the same author a few months previously when he wrote "X-ray diagnosis has been tried on an extended scale. Occasional filling defects have been observed in the caecum, but similar appearances are seen in other forms of dysentery and colitis. Only unsatisfactory assistance can be obtained by this method."

## COMPLEMENT FIXATION REACTIONS.

Such tests have also been used in the diagnosis of amoebiasis. Since *E. histolytica* has not yet been grown in pure culture much of the antigenic material hitherto used has contained bacterial and other products. Nevertheless, there is reason for believing that the reactions may be made specifically to depend on the amoebae. CRAIG (1944) found that of 175 patients giving a positive reaction, 157 (89·7 per cent.) had *E. histolytica* in their stools, whereas of 825 patients with negative reactions, only 12 (1·4 per cent.) had this parasite in the stool. Furthermore, of 6·6 patients giving negative reactions, no less than 220 (32·5 per cent.) were infected with other intestinal protozoa, and no less than 176 (25·1 per cent.) with one or more other species of amoebae. He states that positive reactions become negative in successfully treated patients, usually within 2 weeks after eradication of *E. histolytica*. If a reaction remains positive, even though the stools become negative then infection has not been eliminated and amoebae will reappear in the stools. In such cases further treatment is indicated and should be continued until the reaction becomes negative. Occasionally when a positive reaction became negative as a result of treatment, a return of the positive reaction was noticed before the amoebae could be found in the stools of patients who relapsed. He suggests therefore, that the tests should be repeated at monthly intervals for 3 months in order to assess complete cure. The potential value of such a test in assessing cure is obvious, and the reaction might also help in the diagnosis of amoebomata and liver infections especially with negative stools. Unfortunately however the test does not yet appear one that is either readily applicable or reliable. Dr FULTON kindly examined sera for me from patients with active infections about 0 per cent. showed some degree of complement fixation with the amoebic antigen he used, but he is not yet satisfied about the significance of the results.

## TREATMENT

Turning from diagnosis to treatment, one again encounters much confusion and lack of precision in our knowledge.

## SPECIFIC DRUGS.

The drugs commonly used for the treatment of amoebiasis fall into three groups (1) ipecacuanha derivatives, of which emetine hydrochloride and emetine bismuth iodide are the most renowned (2) iodoxyquinoline compounds, of which the chief are chinlofon and diodoquin, and (3) substituted phenyl arsonates, such as carbamone and stovarsol.

Until recently emetine hydrochloride and emetine bismuth iodide were much less used in the United States of America than in Great Britain, while in the latter country diodoquin was virtually unknown. In Britain chinlofon is usually used by enema whereas in many countries it is often given by

mouth. Such differences strongly suggest that we lack precise knowledge regarding what is the best amoebicidal therapy and how it should be employed.

British practice favours treatment by combinations of the various classes of drugs, a common standard course consisting of emetine bismuth iodide grains 2 or 3 by mouth together with retention enemata of 2½ per cent chiniofon daily for 10 to 12 days followed by carbarsone 0.25 grammes by mouth twice daily for a further 10 days. Sometimes this course is immediately preceded by emetine hydrochloride 1 grain by parenteral injection for a few days. MANSON BAIR (1945) reports that of 600 cases treated by combined drugs only two failed to be cured by one course of treatment and that these two responded to a second course. Unfortunately other workers appear unable to obtain this degree of success and the percentage of cures by the method is variously assessed as lying between 75 and 95 per cent.

#### BACTERIAL INFECTION

Some years ago attention was drawn to the possible part played by con-comitant bacteria in the survival and invasiveness of the amoeba and more recently HARGREAVES (1945) being impressed by the secondary infection of the bowel of a fatal case tried the effect of associating penicillin and succinyl sulphathiazole with the specifically amoebicidal drugs. He observed that oedema and hyperaemia of the mucous membrane subsided and that the ulcers became more superficial when treated with the penicillin and the sulphonamide compound and he stated that "there seems little doubt that in this way the amoebae are rendered more easy of access to emetine." Whether this be true or not it would appear that his view is that emetine remains the ultimate amoebicidal agent. It is difficult therefore to believe that penicillin and sulphonamide can make much difference statistically to the total results of treating amoebiasis, since in general the cases refractory to treatment are not necessarily those with gross lesions. In fact most authors stress the greater difficulty of sterilizing the chronic cyst carriers in whom lesions if present at all are presumably slight.

#### RETENTION ENEMATA

As emetine appears a highly amoebicidal drug it may be asked why it is not used in enemata instead of chiniofon which both *in vivo* and *in vitro* seems relatively less amoebicidal. The explanation usually given is that emetine is too irritant as an enema. I find, however, that a concentration of 1/30,000 appears to give no trouble at all. Such a concentration should have considerable amoebicidal activity, since emetine *in vitro* is active in concentrations of only 1 in 1,000,000 or less, but enemata of 1/30,000 emetine produce results no different from those obtained with the usual chiniofon enemata. For example, two comparable series of patients were treated for 10 days with retention enemata of chiniofon in one group, and of emetine in the other.

each group was also given emetine bismuth iodide by mouth during this 10 days, and a 10 day course of carbarsone subsequently. Of forty-five patients in the group given chiniofon enemata, thirty-one appeared cured three relapsed and the fate of eleven is unknown. Of fifty-three in the group given emetine enemata, thirty-one appeared cured five relapsed and the results of seventeen are unknown. It is of interest to note that when an emetine enema is evacuated after some hours, it shows no amoebicidal property possibly the emetine is completely adsorbed. Further observations suggest that enemata of chiniofon give little amoebicidal advantage in practice although they may have some general antiseptic or cleansing effect. It is possible that enemata may be omitted altogether in the treatment of amoebiasis without loss of efficiency if this be so, a considerable gain in the patient's comfort and a saving in nursing labour could be achieved. It seems therefore a matter worth subjecting to strict experimental test.

#### DRUG RESISTANCE.

To explain cases of amoebiasis refractory to treatment it is sometimes suggested that amoebae may vary in their resistance to the drugs employed, and that inadequate treatment may lead to the development of increased resistance with ultimate failure of the parasite to be influenced by the drugs. There appears little sound experimental evidence to support such a hypothesis in the case of the arsenical and quinoline drugs in amoebiasis and there are good reasons for doubting the validity of many experiments on which the claims for emetine-resistance rest. While there may be scanty scientific justification at present for attributing therapeutic failure to emetine resistance, I have recently obtained, from a patient who has relapsed several times after treatment, amoebae which seem to have a heightened resistance to this drug since Dr Goodwin finds that to inhibit infection in rats with this strain requires ten times the dosage of emetine that suffices to inhibit infection with certain strains from several other patients. Furthermore this patient has continued to pass active *E. histolytica* despite treatment with emetine hydrochloride 1 grain parenterally and emetine bismuth iodide 3 grain orally given together each day for 12 days. At present the disease appears controlled by daily heavy dosage with diodoquin but whether the infection will prove to be completely eradicated or not I cannot yet say. It is obvious that invasion of the liver by this amoeba might have very serious consequences. Similarly suggestive evidence of emetine resistance has been obtained with amoebae from certain other patients who have persistently relapsed after treatment.

#### DRUG METABOLISM.

Other matters on which more precise knowledge is desirable are the exact state and distribution of the drugs themselves in the body. Unfortunately

tests for emetine are insufficiently sensitive to follow the drug adequately in the body, Dr GOODWIN by biological tests and Dr SHARP by chemical means kindly examined a few specimens of blood and other material taken from patients after various doses of emetine, but they found no significant amoebicidal activity. Similarly, amoebicidal activity could not be demonstrated in bowel washouts of patients who were receiving emetine bismuth iodide. Certain speculations, however, may be based upon therapeutic results. For example, emetine, which *in vitro* has such a potent direct action upon *E histolytica*, fails when injected parenterally to cure intestinal infections with this parasite in cats. It has been said that in these animals the drug is largely eliminated in the urine, and it may be that this mechanism prevents the concentration of the drug in the tissues or gut from rising to or being maintained sufficiently long at an amoebicidal level. Alternatively, these animals may metabolize or render impotent the drug in some manner different from that obtaining in man. Even in 1 grain of emetine hydrochloride fail to cure a large proportion of intestinal infections, some placing the relapse rate as high as 90 per cent in chronic carriers the results are said to be particularly poor and many writers seem satisfied to explain this by stating somewhat naïvely that the amoebicidal drug does not act upon cysts, apparently neglecting the fact that the cysts arise from amoebae, and that in these refractory cases the significant point is that these amoebae are escaping destruction and it may be that comparatively small differences in the metabolism or elimination of the drug between individual patients may become manifest clinically by the gross difference between cure and relapse. Save in a small percentage of patients, emetine hydrochloride 1 grain daily appears either insufficient to maintain in the tissues an amoebicidal medium for a sufficient length of time to eradicate the infection or it fails to destroy the parasites in the lumen of the gut and so relapse occurs. Yet this method of treatment appears to have been considered adequate, at any rate until very recently, by many especially in India.

#### EMETINE BISMUTH IODIDE

DOBELL (1917) stated that 3 grains of emetine bismuth iodide daily by mouth for 12 days cured about 90 per cent of carriers of *E histolytica*. This drug is relatively insoluble and stable, save in alkaline medium where it breaks down with the release of emetine, and there are reasons for believing that its ultimate amoebicidal action is due solely to the emetine given by mouth. It would appear, therefore, that an equal amount of emetine given by mouth as emetine bismuth iodide, possibly owing to a slow release of the emetine, maintains an amoebicidal state in the tissues or gut for a more prolonged period, and so more frequently eradicates the infection, than it does when it is given as emetine hydrochloride by parenteral injection. It may not

without significance in this connection to note that when an emetine compound, auremetine, was given by mouth only on alternate days as in the course employed at Liverpool for many years, LASH and ROTSTOV (1945) had a relapse rate equal to 91 per cent. among twenty-six chronic relapsing cases, although it must be admitted that other workers appear to have obtained much more favourable results with this compound and method of treatment. The defects of unduly long spaced doses of such compounds as sulphonamide or penicillin, allowing a fall in the blood or tissue concentration of the drugs, are well recognized, and it may well be that the optimum way of using emetine would be by some method of continuous or intensive administration. In the case of emetine an obvious difficulty arises from the lack of any satisfactory method of determining therapeutic concentrations of the drug in the body and so of controlling the dosage.

The necessity for giving emetine bismuth iodide in a suitable form so that it becomes dispersed in the gut has been much discussed. To obtain a high proportion of cures it may also be necessary for reasons indicated earlier to give, as DODD recommended, 3 grains of emetine bismuth iodide daily and the suggestion of recent years that 2 grains is sufficient may not be without danger. The addition of emetine hydrochloride parenterally in loading doses at the beginning of treatment with emetine bismuth iodide may be reasonable but there seems no certain knowledge on this point. Similarly it seems uncertain whether the addition of chiniofon, carbarsone or similar drugs materially improves the ultimate result. However in the present state of our ignorance and since there appears to be no evidence of any interference phenomenon when such drugs are used together it would not seem unjustifiable to retain their association.

#### ASYMPTOMATIC CARRIERS.

Another question which is frequently argued is whether asymptomatic carriers, for example those in whom cysts are accidentally discovered, should be treated. The cysts arise of course from amoebae within the patient, but whether these amoebae are necessarily invading the bowel tissue or not is uncertain. MANSON BURN (1947) apparently believes in such asymptomatic carriers that the amoeba does not invade the tissues and that it "piles a coprozoic existence, whereas CRAIG (1944) is emphatic that there is no such thing as a healthy carrier if by the term is understood one in whom no lesions are produced. GREENAWAY and CARTER (1937) also held this latter view after examining 2,000 cases of amoebiasis, and believed therefore that all infected individuals should receive treatment. Furthermore, even if one holds the view that asymptomatic carriers have no lesions it would seem reasonable to treat them where practically possible—unless one believes that there are non-pathogenic amoebae indistinguishable from *E. histolytica*—

because no one can foresee when conditions may arise which may render the supposedly temporarily benign amoebae capable of invading the bowel

### RESEARCH

Amoebiasis is a widespread, common and important disease. The majority of sufferers are probably readily curable, but there is an interesting residuum of refractory cases that would well repay scientific study.

Of the so-called "specific" drugs ordinarily used in its treatment none has been in use for less than a decade while the beneficial effect of opacetrinur in dysentery has been known and recorded for several centuries. It is disappointing therefore to find so little precise knowledge and agreement on many of its problems. If I have stressed the divergent views of different authorities and the inconsistencies of even individual authors, it is only to emphasize the extenuating difficulties of the subject and to plead for a further attack upon its problems. In amoebiasis the immediately favourable but often deceptive response to treatment, the disarming but dangerous latency of residual infection, the labour and patience required to assess cure, the lack of suitable susceptible laboratory animals and the limitations of chemical and biological methods for determining amoebicidal drugs in the body, are only some of the factors that conspire to confine our knowledge. The result is that many of our views rest upon imperfectly controlled impressions rather than upon adequate observation, critical analysis and scientific judgment.

### CLINICAL RESEARCH

It would be therefore of the greatest benefit to future work if there were agreed criteria for diagnosis and for test of cure, including methods and number of examinations, and period of observation. It should then be possible satisfactorily to compare different courses of treatment and different responses to a standard treatment under varying conditions. A comparatively small number of cases accurately observed would be more valuable than a welter of material haphazardly handled and uncritically assessed. Since the assessment of the cure of amoebiasis is prolonged, it would be necessary to have patients over whom one would have a long term control. The obvious sources of patients who could be maintained under long observation, would appear to be the Services and the Ministry of Pensions. I suggest, therefore that it would be a good thing for a Centre to be formed to which patients with amoebiasis could be sent. Such a centre in addition to the clinical facilities, should also have suitable protozoological, bacteriological and biochemical laboratories. A small unit of this sort developed near Liverpool after the 1914-18 war and enabled WARRINGTON, YORK and his colleagues to make valuable contributions to our knowledge of the development of amoebae. With air transport there would seem no great difficulty in bringing cases from



any part of the Empire to such a centre. The numbers required would be comparatively small, perhaps a few hundred a year and spread over the United Kingdom and Empire could hardly seriously disorganize military or industrial forces.

#### LABORATORY RESEARCH.

With these clinical investigations there must be closely integrated laboratory studies. When WARRINGTON LORKE seriously commenced chemotherapeutic research, the first 2 years work was largely absorbed in searching for a suitable method for maintaining pathogenic trypanosomes alive *in vitro* at body temperature. This may have seemed somewhat detached and wearisome, but what a rich harvest of chemotherapeutic advances was reaped when once it had been successfully accomplished. Furthermore, during this preliminary period, several facts concerned with the essential metabolism of trypanosomes were investigated and subsequently led to the development of an entirely new series of chemotherapeutic agents. Knowledge of the metabolism of parasites is a most important pass-key to opening the way to their therapeutic control.

In amoebiasis one line of work might be the isolation of amoebae in pure culture. This would involve ascertaining the influence of various bacteria on their development freeing the amoebae from bacteria, and the devising of cultural conditions capable of supporting the amoebae alone. This would undoubtedly throw much light on the fundamental nutritional requirements of amoebae and on their essential metabolism. Once a method were discovered for maintaining amoebae in a bacteria-free fluid medium it is likely that the preliminary chemotherapeutic screening of amoebicidal drugs would be much simplified and the mode of action of several clarified. Following these studies, the influence of the bacterial content or chemical composition of faeces on the pathogenicity of amoebae might well be elucidated.

Studies on the metabolism of the amoebae might also throw light on certain other conditions. For example it seems obvious that the amoebae as mere anatomical entities are not involved in producing the pathological changes of amoebiasis which presumably result from the cytolytic secretions of the amoebae. It is not inconceivable that if this cytotoxin were fully understood some light might be thrown on non-specific ulcerative colitis in which some cytolytic agent of unknown origin possibly metabolic may be involved. It is possible that such knowledge might lead to a completely new approach to the therapy of both conditions.

Another matter for investigation is the fate of amoebicidal drugs in the body and new development. In both chemical and biological techniques may have to be sought. Although the experiment has been very limited I have referred to the failure to demonstrate emetine in the blood of patients under treatment for amoebiasis. If ordinary biological and chemical methods are not sufficiently sensitive it might be possible in the body to trace the drug were

it to contain some radio-active tracer element. Unfortunately it is not possible to synthesize emetine from its simple components and the mere substitution of a tracer element in a side-chain would not necessarily indicate the location and fate of the amoebicidal part of the molecule in the body since breakdown of the molecule might have occurred. Emetine may be produced by methylation of cephrine and it has been suggested that this methyl linkage may remain stable in the body. If this be so then methylating cephrine with for example methyl iodide containing a radio-active isotope of carbon might give an emetine the subsequent fate of which in the body might be traced by means of the radio-active carbon. Whether such a procedure is feasible or practicable is a matter on which expert advice would have to be sought.

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Sir Phillip Manson-Bahr The greater part of my professional life in London for the last 27 years has been devoted to the diagnosis and treatment of amoebic dysentery. I see a great deal of the disease, and am faced all day long with the question "Has this patient got amoebic dysentery or not?" The majority of my cases at present have been in the Services and are sent to me because I am supposed to be able to diagnose this disease and are sent to me have failed to do so. Therefore I am inclined to disagree with some of the statements that have just been made. I have tried to follow-up the patients I have treated. Some, because they are in the Colonial Service, come to me again and again and I have been able to watch them for long periods—an advantage that is not given to everybody. Some die from other causes, others survive, but the great majority of amoebic patients, however chronic, eventually get well. I am not going into a long diatribe about the merits or otherwise of emetine or emetine bismuth iodide. I still consider this the mainstay of our treatment of amoebiasis, whether chronic or acute. When we come to diagnosis, the proctoscope is a useful instrument. It is simple—the simpler the better—and is invaluable. It is advantageous to have a portable light which is introduced for inspection and it is better than a fixed one for viewing the rectal

mucosa. Then there is the Volkmann spoon with a long handle, to obtain scrapings from the rectum. I disagree with the idea that this is not a good method of diagnosis for it often succeeds where other methods fail. Patients are sent to me because other people cannot find amoebae in the faeces. Direct inspection of the mucosa—direct microscopic examination of biopsy material from the mucosa—constitutes the best method of diagnosis. In the great majority of cases the faeces contain neither amoebae nor cysts. Some of the amoebic lesions in the rectum are extremely superficial and difficult to see. Quite unlike the appearances you see in the textbooks they are almost microscopic in size and protean in character. The acute stage looks like ulcerative colitis. There are numerous haemorrhages and the mucous membrane is friable and bleeds when swabbed with cotton wool. Often when scrapings are made from the surface the amoebae are found packed in mosaic like masses. On the other hand the mucous membrane may superficially present an almost normal appearance and all that can be seen are macroscopic bleeding points, from which blood exudes when touched. In one of my cases the patient, an ex-officer had been invalided from the Middle East with chronic diarrhoea. He had been examined many times previously with a negative result. Another early stage is represented by a blotchy discoloration of the mucosa resembling a measles rash and from the hyperaemic areas the biopsies were obtained. In the chronic stage the mucous membrane is beaped up corrugated and pock marked, with pittings resembling a microscopic picture of bomb craters in an aerial photograph. I admit that the minute lesions are not easy to see or distinguish from gland openings which open into the region of the anus.

I am certain that the biopsy pictures are diagnostically important. In the acute stage it is possible to identify the tissue-invading trophozoites of *E. histolytica* moving between the cells and in the more chronic stages to find cysts and pre-cystic stages. I have on one occasion obtained all three stages in a preparation made from a granular patch. In obtaining biopsy specimens it is necessary to scrape the mucosa gently with the Volkmann spoon, but the cutting edge must not be too sharp or else bleeding is excessive. In these preparations I have found other amoebae, such as *Entamoeba coli* *Iodamoeba butschlii* both in the trophozoite and cystic stage, and also ova of *Enterobius*. I believe that some information may be obtained from the consistency and outlines of the mucosal cells. When the outlines and cellular structure are well defined and regular it indicates a normal mucosa, but where these boundaries have been broken down or are ill-defined, it suggests that some pathological factor has been at work. The term "cytogram" is proposed for the cellular picture.

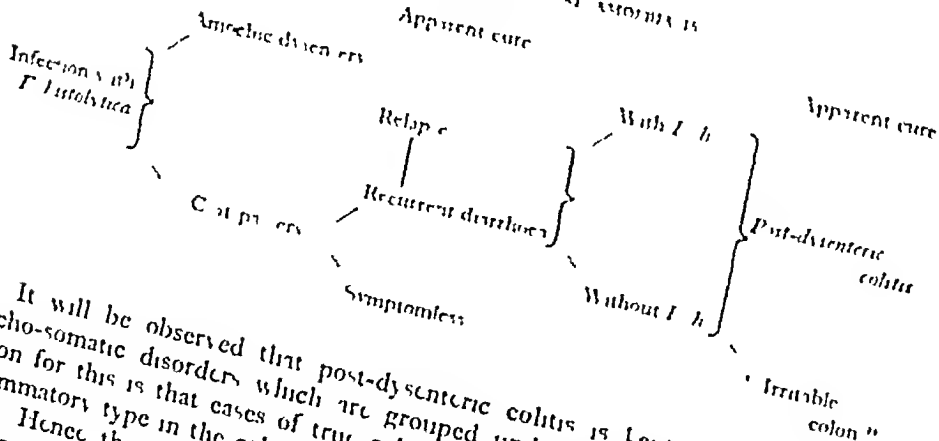
In the preparations containing *E. histolytica* the appearance of the cells approximates the cellular exudate usually seen in amoebic dysentery stools. It is possible to recognize the cells of mucous colitis by the swollen appearance of the goblet cells.

Finally, some reference must be made to radiography as a means of diagnosis. Unfortunately it has little practical value. American authorities have described various filling defects, but in amoebic ulceration of the cecum (or amoebic typhlitis) this method may be of real value. The picture illustrates a peculiar filling effect of the cecum, which at times is a triangular and truncated form which serves to differentiate it from tuberculous retinomycosis or malignant disease. It is in great amoebic colitis that little at a time can usually be obtained by sigmoidoscopy, proctoscopy or examination of the faeces. Therefore in these cases radio copy may be of decisive diagnostic value.

Dr. G. T. Stewart (who gives as a title for his contribution, "Added bacterial infection in amoebic and post-dysenteric colitis") and I think many clinicians would agree that the problem of intestinal amoebiasis at present is twofold. First, there is the presumably straightforward amoebic infection, and, second, there is the possibility of further infection by bacteria. This may be superadded to an existing amoebic infection, or in the relapsing case, and it may persist after the apparent cure of the amoebic infection. The latter group is usually defined as post-dysenteric colitis, though we find it has several features in common with ulcerative colitis and other forms of exudative diarrhoea in people returning from the tropics.

Our observations suggest that amoebiasis follows a clinical course as shown in the following table—

TABLE I  
CLINICAL COURSE OF INTESTINAL AMOEBIASIS



It will be observed that post-dysenteric colitis is kept apart from the psycho-somatic disorders which are grouped under "irritable colon". The reason for this is that cases of true colitis show ulceration and an exudate of inflammatory type in the colon, which suggest an infective basis for the condition. Hence the question of added bacterial infection is highly relevant in such cases.

The term secondary infection in amoebiasis and other forms of colitis has often been used, but I have been able to find very few exact definitions of what is meant by the term. WENTON (1926) indicated the possibility that pathogenic bacteria in the bowel played a role in the development of amoebic dysentery. FRYE and MELNEY (1933) demonstrated that the presence of bacteria could influence the infectivity of the *Entamoeba histolytica* in experimental infections. SHIN LU-CHANG (1945) suggested that the lesions caused by the trophozoites might be rendered more severe by the presence of a virulent intestinal flora. Finally HARGREAVES (1945) reported encouraging results in the treatment of relapsing cases of amoebiasis with penicillin and succinyl sulphathiazole since these substances did not act on the amoeba itself he presumed that they acted on the bacteria responsible for the "secondary infection." The big question, which remains outstanding, is: What are the bacteria producing the secondary infection?

Perhaps some light is shed on this question by Table II, which attempts to illustrate the main qualitative alterations in the bacterial flora

TABLE II  
CHANGES IN THE INTESTINAL FLORA.

	Number in group.	<i>Bact. coli</i>	Non-lactose fermenters.	Paracolon	<i>Strep. faecalis</i>		
					Present	Numerous	Acting as
Controls	61	61 (100%)	11	8 (13%)	42 (69%)	(0)	Nd
Dysenterics	22	22 (100%)	8	8 (27%)	20 (91%)	18 (81%)	3 (13.6%)
Total	83	83	19	16	62	18	3
$\chi^2$ ...				4.97	13.86		
P ...				<0.05	<0.01		

Controls Cases without lower bowel disorders.

Dysenterics Relapsing amoebiasis and post-dysenteric colitis

of the faeces in cases of diarrhoea due to active amoebiasis or post-dysenteric colitis. These are compared with control cases, in which acute diarrhoea or any disorder of the lower bowel is excluded. It will be seen that *Bact. coli* is equally prevalent in both groups. Paracolon bacilli and *Streptococcus faecalis* are more prevalent in the dysenteric group and statistical analysis suggests that this increased prevalence is unlikely to have arisen by chance.

Some further details concerning these bacteria may be of interest.

*Bacterium coli*. This organism and its biochemical variants are ubiquitous in normal and dysenteric cases alike. As often as not, it cannot be agglutinated by the patient's own serum, even when the colon is undergoing inflammatory

changes. On the other hand, its capacity for extra-intestinal pathogenicity is well known, e.g., in urinary infections and peritonitis, and it is possible that it may become pathogenic in the tissues (as opposed to the lumen) of the colon.

**Paracolon Group.** The diverse members of this group have been studied recently by SEVITT, who showed that 75 per cent of Group 1 strains were serologically identical (Type A). Many of these organisms were isolated from cases of infantile diarrhoea and enteritis and produced experimentally a similar enteritis in kittens. SEVITT considered that Group 1 (Type A) strains were therefore pathogenic, or facultatively so. It may be noted that Group 1 bacilli are related biochemically to the salmonellas and that they share minor antigens with the dysentery bacilli.

***Streptococcus faecalis*** The increased prevalence of this organism in the dysenteric group is not easy to explain. It may be that it grew more readily in the liquid medium of a diarrhoeic stool but in some cases it attained a predominance not often seen in the normal subject. The strains isolated were not biochemically uniform and did not produce a haemolysin. DIBLE (1921) showed that this organism was not usually pathogenic to mice, and our strains did not appear to be pathogenic to guinea-pigs or rats. The organism is capable, however, of assuming extra-intestinal pathogenicity in the human.

***Aerogenes*** The main importance of this organism is its ability to overgrow other bacteria during the acute phase of amoebiasis when the trophozoites are most active. Cultures of the organism are readily lethal to animals and it shows pathogenic activity outside the bowel.

The evidence which I have adduced is far from conclusive, but it may throw some light on the mechanism of "secondary infection" or, as I prefer to call it, "added bacterial infection," since it may be a factor in the pathogenesis of amoebiasis. The major role, however, is probably in late relapsing cases and in post-dysenteric colitis where the exudate becomes frankly purulent.

If the hypothesis of added bacterial infection is accepted, then rational chemotherapy becomes feasible. I should like to mention briefly some trials we have made in this connection.

*In vitro*, penicillin inhibits strains of *Bact. coli*, paracolon and *S. faecalis* at concentrations ranging from 20 to 100 units per c.c.m.—much higher than is usually attained in the tissues. In the presence of certain sulphonamides, however, a synergic action obtains whereby the effective bacteriostatic concentration of each drug is considerably lowered and may come within the therapeutic range of parenteral or regional therapy. I have described these *in vitro* tests in detail elsewhere and we have found also that the requisite concentrations of the two substances are attainable in the human subject.

With regard to clinical trials, SIR HOWARD FLOREY and Professor MAEGRAITH thought that it would be of interest to see if the penicillinase-producing members of the intestinal flora could prevent the action of penicillin. By putting this question to the test we found that penicillinase-producing bacteria were often

completely absent in their absence, penicillin could remain active in the presence of other coliforms or as Dr O CONNOR will show in the lumen of the bowel. We have therefore given penicillin parenterally with sulphathiazole orally to some patients and penicillin rectally to others. There is not time to supply the clinical details but we formed the impression that encouraging results may be obtained. I have in mind two cases, both of which had been resistant to prolonged rest and to other forms of treatment, in which the clinical results were striking. Correlated with this, we have noted various degrees of suppression of *S. faecalis* and other intestinal bacteria. But I must mention also another case in which combined therapy with penicillin and sulphathiazole gave no improvement. Failures such as this are probably to be expected in dealing with infections by mixed bacteria whose resistance to the bacteriostatic agents varies both in nature and in degree. With that reservation penicillin, sulphonamides, quinoxyl and, possibly streptomycin would appear to have a logical place in the treatment of added bacterial infection in relapsing amoebiasis and post-dysenteric colitis.

It is a pleasure to acknowledge the co-operation of Professor MACGRAITH, Dr ADAMS, and the staff of the wards and laboratory at the School of Tropical Medicine and at Smithdown Road Hospital, Liverpool.

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Dr R. J. O'CONNOR. When the staff of the Liverpool School of Tropical Medicine used penicillin, administered rectally in the treatment of amoebiasis, I was asked to trace the fate of the penicillin. Six cases were investigated, all of which were passing three to five motions a day containing varying amounts of blood, pus and mucus. One million units of penicillin were administered each day in a single, small retention enema and treatment continued for 10 days.

In investigating the degree of persistence of penicillin in the bowel it was found that the first motion passed contained a large amount of penicillin at a high concentration and subsequent motions contained little or none. Thus it could be taken that the amount of penicillin in the first motion after administration was approximately the amount of penicillin remaining in the bowel at the time of passage of that motion. The results obtained were sufficiently constant to be indicated by averages derived from the six cases over the whole period of treatment and these are set forth in Table I, where it will be seen that about half the dose was recovered at 6 hours, and about one third at 14

TABLE I

PENICILLIN IN FIRST MOTION PASSED AFTER 1,000,000 UNITS RECTALLY

Time of motion (Hours)	Concentration Units/c.c.	Total amount Units
6	4,000	500,000
10	5,000	400,000
12	3,000	350,000
14	2,000	350,000

hours The high concentration of penicillin in the motions should be noted as it suggests that there has been little diffusion of the penicillin into the more proximal portions of the bowel

In order to estimate the absorption of the penicillin, serum blood levels were estimated every 4 hours, but these were very variable Although some penicillin was usually demonstrable in samples taken during the first 12 hours, the concentration was never more than 0.3 unit per c.c., and always much less than the levels obtained by the intramuscular injection of much smaller amounts

It was therefore found much more satisfactory to estimate absorption by measuring the urinary excretion, and daily averages for four of the six cases are detailed in Table II The total amount excreted ranged from 5,000 to 100,000 units a day, with a peak of excretion at about 6 hours These figures

TABLE II

URINARY EXCRETION OF PENICILLIN AFTER 1,000,000 UNITS RECTALLY AT 0 HOURS  
(Average over 10 days)

Time	Case 1		Case 2		Case 3		Case 4	
Hours	Conc. U/c.c.	Total units	Conc. U/c.c.	Total units	Conc. U/c.c.	Total units	Conc. U/c.c.	Total units
2	3	900	8	800	37	10,100	58	15,100
6	6	2,800	28	9,200	32	14,400	107	45,000
10	5	1,900	13	3,400	23	10,800	90	28,700
14	tr	10	16	2,300	12	2,500	36	7,000
18	—	—	2	1,100	1	200	4	1,800
22	—	—	1	500	tr	100	3	1,100
24	—	—	—	—	—	—	1	50
Total		5,600		17,300		38,100		98,750



suggest the possibility that, by the rectal administration of penicillin, urinary levels of some therapeutic value might be obtained.

The effect of penicillin on the stools of the six cases is shown in Table III where it will be seen that there has been improvement in Cases 2 and 4. A detailed investigation of the bacterial flora was not attempted but a rough quantitative estimate of the number of organisms of the enterococcus group was made in three cases and a temporary suppression during treatment demonstrated. This organism, *in vitro* required about 20 units per c.c. to prevent its growth.

TABLE III.  
CHARACTER OF FAECES BEFORE AND AFTER RECTAL ADMINISTRATION OF  
1,000,000 UNITS PENICILLIN DAILY FOR 10 DAYS.

Case.	Before treatment (1st day).		After treatment (10th day).	
	Number	Character	Number	Character
1	5	Fluid Mucous	3	Fluid Mucous
	3	Fluid Mucous	1	Solid
2	2	Semi-solid Blood	4	Semi-solid Blood, Pus
4	4	Fluid Pus	1	Semi-solid
5	3	Fluid Blood, Pus	4	Fluid Pus
6	5	Fluid Mucous, blood, pus	4	Fluid Pus

It is realized that these investigations are too superficial to bear any weight of deduction but it appears that if 1,000,000 units of penicillin were administered daily by the rectum in two or three divided doses it would be possible to maintain a high concentration continuously in the rectum and this might be expected to modify the bacterial flora.

Air-Commodore T. C. Morton. I propose to confine my paper to cases of typical amoebiasis encountered during the recent war—the difficulty I am faced with is how to select my cases in the time at my disposal.

## FULMINATING AMOEBIC DYSENTERY

This is extremely rare and only occurs in war or under famine conditions

*Case 1*—In September, 1941, a soldier was admitted to an R A F hospital in Iraq from a convoy with a diagnosis of an acute abdomen. He was pulseless and collapsed, dehydrated and vomiting, the abdominal wall was guarded and exquisitely tender. After I V glucose saline and a blood transfusion, the patient rallied sufficiently to give a history. He had left England 3 months previously by sea, he had gone ashore for a few days in Durban, and *en route* for Bombay developed a sharp attack of diarrhoea which had been successfully treated. He was 3 weeks in a rest camp in Bombay and the diarrhoea recurred. He did not report sick as he was afraid of being separated from his friends in his county regiment. He hoped to carry on with his battalion until they had reached their final destination, when he intended to report sick. He transhipped to Basrah, by this time he was passing blood and mucus some three to six times a day. On disembarking at Basrah, he proceeded by road in an open truck from Basrah to Habbaniyah. Unable to eat bully beef and biscuits, his only sustenance was frequent sips of water. Four days were spent on this desert trip with a shade temperature of at least 112° F, and his sufferings can only be imagined. He was transferred to our hospital in a collapsed moribund state. There had been no diarrhoea for the last 3 days and auscultation revealed a silent abdomen. On rectal examination, some dark blood-stained faecal material was present on the glove and oozed from the anus. Operation was not considered justifiable until he had rallied sufficiently, but death supervened within an hour. A provisional diagnosis of fulminating amoebiasis was made. A postmortem examination was carried out personally within half an hour of death. On opening the abdominal cavity a general peritonitis was evident with a recent perforation in the caecum where an area of the bowel wall had sloughed away and there was a second perforation in the descending colon. The whole of the large bowel from caecum to anus was necrotic, a purplish green in colour and of the consistency of wet disintegrating blotting paper. On opening the bowel the interior was a dark purplish diffuent homogeneous slimy track, no individual ulceration was distinguishable. There was no evidence of any thickening of the caecum and the bowel wall was too friable to attempt to remove it *in toto*. The liver appeared to be normal macroscopically, but I regret to say it was not sectioned. Scrapings were taken from the lumen of the bowel and on examination were found to be literally crawling with *Entamoeba histolytica*. The most interesting feature of this case is that at the outside limit the disease could only have been contracted 6 weeks previously in Durban and in all probability it had been contracted later in Bombay.

## AMOEBOMA OR AMOEBIC GRANULOMA SIMULATING A NEOPLASM

This may be defined as a hyperplastic localized tissue reaction of the colon giving rise to a palpable growth in the abdomen or rectum or both and

liable to be mistaken for a neoplasm. It is relatively rare, but I have encountered seven cases during the recent war.

*Case 2.*—A sergeant, aged 41 was seen in 1945. *Previous history* 3 months previously on a flight from Cairo to India, he developed a severe diarrhoea which cleared up spontaneously in 2 days. Diarrhoea has alternated with constipation ever since. For the past 2½ months he has had epigastric pain, together with distension of the stomach and spells of diarrhoea and constipation. He developed a moderate watery diarrhoea with light stools five days before he was admitted to an American military hospital in Paris. On examination he was found to be tender on the right side of the abdomen at the level of the umbilicus—no mass was palpable. His total white cell count was 8,800 with 70 per cent. polymorphonuclear leucocytes. His B.S.R. was 30 mm. in 1 hour. Four specimens of faeces were negative for *E. histolytica* and on culture. Barium meal negative. Kahn test was positive. 11 days later he was transferred by air to the United Kingdom and was admitted to an R.A.F. hospital.

On examination, a firm rounded mass could be felt just above the right iliac fossa which extended upwards towards the liver and posteriorly towards the loin. It was very tender and did not move on respiration. No enlargement of the liver. X-ray of the lungs and diaphragm normal. Stools were liquid and greenish in colour with small plugs of blood-stained mucus. Blood count showed a total white cell count of 17,000 per c.mm. with polymorphonuclear leucocytes 88 per cent. Wassermann and Kahn strongly positive. Rectal examination very tender and a large polypoidal cauliflower mass could be felt. Proctoscopy revealed an irregular ulcerated mass just beyond the internal sphincter—scrapings from the ulcers were full of active *E. histolytica* containing red blood corpuscles. A barium enema next morning showed gross irregularity of the rectum and pelvic colon with one filling defect on the left wall of the rectum. Some barium did enter the transverse colon showing it to be very irregular in outline. The caecum and adjacent ascending colon contained a thin streak of barium only—either due to spasm or filling defect. The tender tumour palpable in the R.I.F. corresponds with this caecal filling defect. The patient was given 1 grain emetine intramuscularly for 12 days. For the first 7 of these days he was given 2,500,000 units of penicillin and diodoquin was started on completion of the course—three tablets t.i.d. for 25 days. In 6 days the large mass above the right iliac fossa was no longer palpable. Its rapid disappearance is difficult to explain unless the mass was due to the great omentum guarding a threatened perforation of the ascending colon. The diarrhoea had ceased and only two stools were passed in 24 hours, the patient was cheerful and eating well. Seventeen days later a proctoscopy revealed a mass the size of a tangerine orange, which appeared to completely obstruct the rectum but possessed a lumen admitting the tip of one finger.

Three weeks later palpation revealed a fibrous band at the site of the

amoeboma which had completely disappeared, the band could be felt under the mucosa extending upwards

The patient had gained 2 stone in weight in the last 5 weeks. Barium enema the whole large intestine fills easily with barium. In the rectum, about 2 inches from the anus, there is a little wasting of the lumen.

A course of 12 days E B I and yatren were given and the patient was sigmoidoscoped in 3 months' time to 25 cm. and a normal mucosa was found. He was in excellent health, but was still under treatment for syphilis.

#### AMOEBIIC ABSCESS OF THE LIVER

One case presented such unusual features that it is well worth recording. I can find no record of a similar case in the literature and it is reasonable to presume that the peculiar allergic attacks he suffered from were due to the leakage of the contents of a liver abscess into a small vein.

*Case 3*—First attack of amoebic dysentery, 1943, and nine clinical recurrences, 1943, to November, 1944. All symptoms subsided from November, 1944, until April, 1946.

#### *Present illness*

*22nd April*—Complained to civilian practitioner of pain below the left costal margin. Was regarded as having pneumonia but blood was noticed in the stools.

*27th April*—Seen at a civil hospital, where *E. histolytica* cysts were found in the stools after 3 weeks' stool examinations. During this period he experienced a generalized urticarial rash, with a sudden malaise and dyspnoea lasting for 2 or 3 days. He was given emetine 10 grains, E B L, and yatren and stovarsol, which relieved the symptoms previously described as pneumonia and he was up and about the hospital when, early in June, he experienced a sudden, severe, upper sub-sternal pain which passed to the right costal margin, and was followed immediately by an urticarial attack lasting several days, associated with vomiting and dyspnoea. He was given nine injections of emetine with no relief, though the urticaria improved. He was admitted to us for further treatment on 20.7.46. Temperature 100, pulse 106. Complained of pain in right costal margin.

#### *Examination*

Tender in right costal margin where resistance was felt, but no liver edge was clearly defined.

*White Blood Count on Admission*—19,300, with 76 per cent polymorphs.

*Chest X-ray*—Showed clear lung fields with normal diaphragmatic movements.

*Stools*—Showed no pathogens.

*Sigmoidoscopy*—Normal mucosa.

24.7.46—Sudden onset of urticarial rash over forehead and trunk, associated with a very collapsed condition. Adrenalin hydrochloride 10 mm. 1/1 000 L.M. Given calcium gluconate I.V., and condition improved.

*Casosa Test*—Negative. Amoebic complement fixation test negative. Hydatid complement fixation test negative.

*Diagnosis*—Based on general grouping of symptoms, together with the history was amoebic hepatitis with a possible secondary infection. Was given 1,500 000 units of penicillin and 30 grammes sulphathiazole starting 26.7.46. Course completed in 4 days.

*White count* fell to 7,200 then started to rise to 13,300. Course of emetine started and 6 grains given.

*Wassermann and Kahn*.—Negative.

During this time, discomfort at the right costal margin had considerably improved with penicillin and sulphathiazole but the temperature was still raised and an exploratory liver puncture was carried out on 7.8.46. No abscess cavity found. 2 days later experienced further urticarial rash on flexor surface of both forearms followed by increased abdominal discomfort and vomiting.

*White blood count*—11,200 on day of liver aspiration, rising to 33,000 2 days later. His eosinophils were absent on the day of his allergic attacks but appeared 2 or 3 days later in moderate numbers. They never exceeded 6 per cent.

15.8.46 at 20.00 *Hourly*.—Operation. Abscess of quadrate lobe of liver found.  $\frac{1}{2}$  pint of brown anchovy like pus aspirated. The abscess tube drained well.

24.9.46—Well. No symptoms.

Seen 2 months later perfectly fit. No recurrence of urticarial attacks after operation.

#### AMOEBIASIS SIMULATING APPENDICITIS WITH AN UNUSUAL COMPLICATION.

This is a relatively common syndrome but the following case is probably unique.

*Case 4*—In 1947 the patient was under treatment for subacute amoebiasis. Active *E. histolytica* were present in his stools with mucus but no blood. He had received two injections of emetine when he developed acute pain in the right iliac fossa and was tender on palpation over McBurney's point. A surgeon who was called in advised immediate operation but in view of the history I felt it was advisable to defer operation until at least 4 grains of emetine had been given and to hold a watching brief for the time being. The pain, however, increased in severity. It was of a constant character with acute colicky exacerbations. On the 5th day an operation was carried out and a large swollen appendix was removed. On sectioning it, to my great surprise I found part of

a large ascaris occupying the proximal half of the appendix, the surgeon having unwittingly performed a combined appendicectomy and decapitation  
 [A section from this case was shown under the microscope to convince the incredulous]

## TREATMENT

I did not intend to say anything about treatment, but I would like to mention a course of treatment we have found invaluable in very chronic relapsing cases with blood and mucus. We call it the Special Halton Treatment it consists of penicillin 15 mega units by needle + 100 grammes of sulphasucidine for the first 5 days, followed by 1 grain of emetine by needle for 6 or 10 days, the latter dosage being reserved for cases in which there is a markedly raised total white cell count, together with the emetine, the patient receives 3 tablets t d s of diodoquin for 21 days, by mouth. This is followed by emetine eneseales (Eli Lilly & Co), 2 tablets of  $1\frac{1}{3}$  grain each t d s, for 10 days, together with a yatren retention enema of  $2\frac{1}{2}$  per cent yatren commencing with 250 c c and going up to 700 c c. This has proved to be effective in every one of our residual resistant war cases up to date and the course has been extremely well tolerated.

S/Ldr R DALY has conclusively proved by barium enema of the same consistency of the yatren that with 200 c c one only gets to the splenic flexure, with 500 c c to the hepatic flexure and that it is necessary to use 700 c c to ensure filling the caecum.

Finally, I feel that it is our duty, by constant reiteration in the medical journals, to bring to the notice of the medical profession at home the necessity of excluding amoebiasis before embarking on any surgical operations on the intestinal tract or ano-rectal region in a patient who has been in the tropics.

Mr L G Goodwin (who later showed a short film recording a method of producing experimental infections with *E. histolytica* in young rats) said As long ago as 1932 MELENEY and FRYE described the infection of kittens by inoculation of cultures directly into the caecum, and DESCHIENS and PROVOST 5 years later wrote an illustrated account of the procedure. Last year, at the meeting of this Society in Liverpool, Mr W R JONES demonstrated the application of the method to young rats, and has since published a full account in the *Annals of Tropical Medicine*. The methods we have developed at the Wellcome Laboratories of Tropical Medicine differ somewhat in detail from those described by Mr JONES, but the underlying principles are the same. Before I show the film I would like to draw your attention to the fact that besides being useful for the examination of new drugs, the experimental infection in the rat may help to solve some of the knotty problems mentioned tonight.

For example, we have been able to show that strains derived from different sources vary in their infectivity for the rat, and that pathogenicity does not

depend upon the number of amoebae injected, nor upon the severity of the human infections from which they were derived. We have shown recently that a strain from a symptomless carrier will produce severe lesions in the cecum of the rat, thus confirming the similar observations upon kittens already mentioned by the PRESIDENT.

With regard to emetine resistance, I cannot agree with Dr MURRAY ROYD that we have demonstrated this conclusively. It is true that the strain from his patient required doses of emetine within the toxic range to produce any effect upon infections in the rat but on the other hand, the most sensitive strain we have handled was derived from one of Air Commodore MONTGOMERY'S patients who had also received many unsuccessful treatments with emetine. This evidence adds weight to the view that pathogenicity and drug resistance may not be intrinsic properties of a strain of amoeba but may be determined by other factors such as the environment provided by the host and his particular way of dealing with the drugs administered, and the nature of the bacterial flora accompanying the strain.

It is a pleasure to acknowledge my indebtedness to the staff of the Wellcome Film Unit, and especially to Mr DOUGLAS FISHER, who photographed and prepared the film.

[Mr FISHER then projected the film to which Mr GOODWIN had referred.]

Dr L. E. NAPIER: Mr PRESIDENT it is now very late, and you yourself discussed the "carrier" question, the only point to which I really wanted to draw attention tonight so I will be brief. There does not appear to be any accepted teaching on this subject. Many people are ignorant that there is such a thing as a high percentage of cyst passers in temperate countries. You mentioned the figure of 20 per cent. for America. That estimate was made by FAUST and I think it was high one 10 per cent. would be a more conservative estimate. This 10 per cent. refers to a cross section of the population at one time. You yourself were only a carrier for a few years, so that if at any one time 10 per cent. are carriers, it looks as if 50 per cent. or more will be carriers at one time or another during their lives. It is hardly to be believed that all these people have ulcers in the intestines or still more so that this high percentage of persons in the United States are in a state of sub-health on account of this infection. You in your excellent opening of this discussion, sat on the fence, but I think the time for the medical profession sitting on the fence is over and we should try to get down to the problem of whether in a non-tropical country the carrier means anything or not. Other wise, in a very short time in the lay press there will be advertisements on these lines "One in five have it! Do you feel in perfect health? If you don't you are probably a carrier of *Entamoeba histolytica*. Take our pills for 21 days and you will be cured and feel a different man. Dr MURRAY ROYD said—and I believe that you agreed with him—that if he met person who was a

carrier he would feel that it was his duty to cure him. But you say we should not look for carriers. That is, in my opinion, not the correct scientific outlook.

When I was in America I tried to persuade them to take 1,000 men going into the Army who were cyst-passers, not to treat them or even tell them, but to watch their Army careers and compare them with those of 1,000 men who had no cysts in their stools. This would at least be one way of settling whether the infections in these men had a detrimental effect on health. Some such investigation might be carried out with recruits in this country.

**Dr C A Hoare** I propose to deal with certain aspects of the host-parasite relationship in amoebiasis and particularly with the mode of life of *Entamoeba histolytica* in the human host, regarding which there is considerable controversy. The majority of workers believe that this amoeba invariably invades the intestinal wall, and that it feeds exclusively on elements of the host's tissues and on red blood corpuscles. While there can be no doubt about the behaviour and food habits of *E. histolytica* in clinical cases of amoebiasis, its mode of existence in symptomless and chronic infections stands in need of revision. In the past a number of observers have demonstrated that in symptomless carriers the amoebae live on the surface of the mucous membrane and among the contents of the gut, where they feed on bacteria and possibly on faecal debris. From these observations it would appear that in carriers, and among the contents of the gut, where they feed on bacteria and possibly on faecal debris, *E. histolytica* is capable of living as a commensal without necessarily damaging the host's intestine. As you have heard, this view is far from being generally accepted. Thus CRAIG and FAUST (1945) deny the very existence of healthy carriers. This view is based chiefly on postmortem examinations carried out by FAUST (1941). As already mentioned by the PRESIDENT, among the thirteen persons harbouring *E. histolytica*, only seven showed minute or superficial lesions of the mucosa, but FAUST assumed that undetected lesions must have been present in the remaining cases as well. However, the only permissible conclusion from these findings is that in about 50 per cent of the cases of symptomless infection observed by him the amoebae actually occurred in the absence of any lesions. Other workers, notably BRUMPT (1936), though fully aware that in carriers *E. histolytica* may lead a commensal life and ingest bacteria, evaded the issue, suggesting that such infections are due to a distinct species (*E. dispar*). I have recently examined a number of faecal preparations from cases of chronic amoebiasis who have been passing cysts and active amoebae, and I was able to satisfy myself that a certain proportion of the amoebae had food vacuoles containing bacteria. Further light has been thrown on the food habits of *E. histolytica* by its behaviour in cultures and in lower animals. As is known, in cultures the amoebae feed on bacteria and on starch. In natural and experimental infections of macaque monkeys, *E. histolytica* behaves in most cases as a true commensal, living in the lumen of the gut and feeding on bacteria and faecal debris, while in experi-



mentally infected rats the amoebae may live as commensals and ingest bacteria or invade the intestinal wall and feed on red blood corpuscles. It is thus seen that the food habits of *E. histolytica* vary according to its mode of existence in the bowel. That *E. histolytica* is omnivorous is also evident from the fact that its food habits can be changed at will. Thus when amoebae, which have been living as commensals and feeding on bacteria in the gut of a monkey are transferred to a kitten, they invade its intestinal wall and start feeding on red blood corpuscles, but if the amoebae from an infected kitten are recovered in culture they revert to their original food habits and ingest bacteria. Though the question regarding the behaviour of *E. histolytica* in the human bowel stands in need of further investigation, the position of those who regard it as an obligatory tissue parasite is untenable even on *a priori* grounds, for it is inconceivable that an organism feeding exclusively on tissue elements and erythrocytes in the human host should completely change its habits in other mammalian hosts and in culture, and become a feeder on bacteria. However such changes find a ready explanation if it is assumed that amoebic infection shows every gradation from commensalism to true parasitism. In accordance with this view the host parasite relationships in amoebiasis can be visualized as follows. Although *E. histolytica* is a pathogenic parasite, in the sense that it is endowed with the power of invading the tissues of its host, producing lesions and clinical symptoms of disease, in the great majority of cases it does not manifest any evidence of virulence, for in about 90 per cent. of persons harbouring this parasite the infection is symptomless and the host is a carrier. In some of these carriers the amoeba lives as a harmless commensal, without invading the tissues of the gut, though it is conceivable that it might do so at some period of the infection. But even when the gut wall is invaded, in most cases the reparative powers of the host are capable of restricting the damage, with the result that no clinical symptoms are revealed, and only in a minority of cases is the host's defence broken and the disease develops unhampered, with the well-known symptoms of amoebic dysentery. The existence of a commensal phase in the life history of *E. histolytica* has a direct bearing on the epidemiology and therapy of amoebiasis. In this connection it may be noted that some authors maintain that the amoebae living in the lumen of the gut are the only forms capable of encysting and propagating the infection, while others have found that these forms are more resistant to the action of emetine than the tissue forms. However these views stand in need of verification.

Dr H. R. Andrew May I make two very brief remarks one concerns the *E. histolytica* cyst rate amongst Australian troops. In Australia, Major LANCASTER examined 155 healthy troops and the percentage rate was 3.2. Amongst the troops in New Guinea 569 were examined and the rate was 3.7 per cent. These numbers are not very different. What was different was that in New Guinea there was a high bacillary rate and an appreciable rate

of amoebic dysentery. One cannot help feeling that the likely explanation for the occurrence of considerable numbers of overt amoebic infection in New Guinea, as opposed to its rarity in Australia, lies in the wounding of the mucosa caused by pathogenic bacilli.

Another interesting point which came out of that area was the result of a postmortem examination on a Japanese prisoner of war by Dr E H DERRICK. He found lesions in the stomach, colon, lungs and brain but not in the liver. The sections were examined by a number of pathologists, including our PRESIDENT and Brigadier N HAMILTON FAIRLEY. *Iodamoeba butchli* were present in large numbers. We followed up some *I butchli* infestations, and four had symptoms which could have been due to chronic amoebiasis. These were treated with EBI with indifferent clinical results. Two were better, two were no better. One feels in the light of the one striking case which was fatal that the problem still is *sub judice*, and it may be that *I butchli* is a rare but definite cause of amoebiasis.

Dr H S Stannus. Future work in the treatment of amoebiasis should surely lie along the lines followed by WARRINGTON YORKE, as mentioned by Dr MURGATROYD.

The problem is one of denying to the amoeba some one or more of the nutrients essential for its metabolism and multiplication, just as the sulphonamides deny para-amino benzoic acid to *B dysenteriae*.

Some of the evidence brought forward this evening suggests that the amoeba only thrives in the presence of a bacillary dysentery infection. Is it possible the bacillary infection is responsible for the biosynthesis of nutrients or vitamins essential for the amoeba?

Are the feeding habits and food supplies different in the two cases when the amoeba lives in the bowel lumen and when it invades the tissue?

Is there any information of interest on the infectivity of laboratory animals fed deficient diets or diets supplemented in various ways?

Mr L G Goodwin. We have done some work with vitamin-deficient rats, in collaboration with Mr A L BACHARACH. We found very little difference between the susceptibility of the deficient and the normal rat. The deficient rat was, however, much more susceptible to *E muris*, the amoeba which commonly lives in the caecum of the wild rat, and the presence of this organism confuses the picture.

Mr W Richard Jones. It is generally accepted that progress in the chemotherapy of amoebiasis has been hindered by the lack of an experimental infection suitable for the large-scale screening of possible chemotherapeutic agents. Now that this defect has been remedied we might reasonably expect progress to be made in the disease. For a number of years I have been working

on the application of the experimental infection of rats with *E. histolytica*, and there are one or two points I should like to raise. Before doing so, I congratulate Mr Goodwin on the very fine film he has just shown to us. The experimental infection differs widely from the blood stream infections used in malaria and trypanosomiasis research. It can be influenced by numerous factors other than the test drug, and considerable caution has to be exercised in interpreting results. It is essentially an acute infection and is transient. In this respect it differs from that phase of amoebiasis which causes most trouble to clinicians, namely the chronic, cyst-passing phase. This at first sight may appear a disadvantage, but whatever the disadvantages of the method I have been able to make good progress in the experimental chemotherapy of the disease. It would be premature to discuss the results I have obtained now and from the clinicians point of view this may be disappointing after all, they are not so much interested in a new method as in a new drug arising from the method. I was going to mention some of my results in the experimental evaluation of known amoebicides but as they conform so closely to Mr Goodwin's results I need not discuss them in detail. I examined the therapeutic effect of emetine, chinofon, carbarsone, stovarsol and diodoquin and found, as Mr Goodwin found, that emetine and chinofon were by far the most effective of the series, with stovarsol, carbarsone and diodoquin a long way behind. Incidentally I found diodoquin the least effective and only by giving repeated doses of this drug was I able to demonstrate any action at all.

Mr Goodwin Although large amounts of diodoquin are required to cure rats, the chemotherapeutic index is not determinable, since it is almost impossible to kill a rat with it. This is a point in its favour.

Dr W. E. Cooke During the war when we had not the help of our hospital and so were unable to examine stools frequently in patients, where the first stool examination proved negative, proctoscopy and examination of the material so obtained often showed amoebic infection. Indeed, on a few occasions the use of an ordinary finger stall and examination of the faeces so obtained showed *E. histolytica* present. This procedure may be helpful when a proctoscope is not available.

Dr Wanyon (in reply) There is no doubt we have had a very interesting discussion this evening, and a number of points requiring thought and consideration have been raised.

There is always the troublesome question of the healthy carrier. It has to be admitted that a carrier may at any time suddenly develop amoebic dysentery or even liver abscess without any dysentery whatever. This has led some to consider the possibility of discovering all carriers and submitting them to treatment in order to eradicate the infection. In the early part of the

1914-18 war some attempt was made to discover the carriers but it soon became evident that there were so many of these in the Forces that it was quite impossible to isolate and treat them all. The plan was abandoned and treatment was applied only to those who reported sick and were found to be clinically suffering from an amoebic infection. I think there can be no doubt that the majority of those with amoebic infection do not suffer in any way and that if re-infections do not occur the existing infection dies out in the course of a year or two. On the other hand, there are some who regard the carrier position very seriously, the extreme view having been well described by ALBRIGHT and GORDON\* in their recent paper on the "Present Status of the Problem of Amebiasis" in the U.S.A. They wrote —

"We cannot emphasize too strongly our clinical impression that the 'carrier' represents an active stage of the disease, often, if not regularly, completely free of symptoms, and for this reason the most easily overlooked and neglected. It is the stage of the disease when the opportunity is greatest for prevention of serious and even fatal complications. It is the time when the best chance exists for complete eradication of the infection and for restoration of the patient to full health in the shortest possible time, with the lowest probability of troublesome sequelae. Surely no one would reserve definitive treatment in tuberculosis until cavitation developed. In amebiasis, as in tuberculosis, the time for full and complete therapy is as early in the course of the disease as it is possible to give it. This will be, in the vast majority of instances in the United States, in the stage represented by the asymptomatic carrier."

If this view is correct it would seem that no carrier should be left untreated, but how is this to be undertaken in a country in which the general carrier rate is something like 10 per cent of the entire population and where the chances of re-infection will be the same as they were before?

\* ALBRIGHT E C & GORDON, E S (1947) Present status of the problem of amebiasis, *Arch intern Med*, 79, 253



## COMMUNICATIONS

### STUDIES ON THE GENUS *SHIGELLA*

BY

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#### I ANTIGENIC STUDIES BY THE PRECIPITATION REACTION

Investigators of the last decades showed great interest in the study of the antigenic structure of the *Shigella* group of organisms. ANDREWES and INMAN (1919) conceived a multiplicity of antigenic components represented within the body of the bacillus, like a spectrum, having a quantitative predominance of the factor that determines the type. However, their opinion was challenged by BOYD (1940) who stated that each type is characterized by a type-specific antigen, and all types (V, W, Z, and three new types introduced by BOYD, 103, 119, and 88 Newcastle) are inter-connected by a common antigen specific to the species. BOYD's work was later confirmed in its main points by WHEELER (1944). The common antigen of the species was analysed by the latter and found to consist of nine components. WHEELER agrees with BOYD in that there are six type specific antigens, one in each organism which characterizes them.

The latest attempt to solve the already complicated situation concerning the antigenic architecture of the members of the genus *Shigella* is the work of WEIL, BLACK and FARSETTA (1944). These workers believe that the main problem for the classification of the group lies in the essential antigens that dominate the immunological reactions of the host, which are the type antigens, and not the group specific antigens of the species. They also believe that the problem of the antigenic structure of the *Shigella* group and that of the genus *Salmonella* are analogous.

WEIL proposes a new classification for the genus *Shigella* based on the primary or dominant antigens. This new schema comprises all of BOYD's Flexner types, including the rare strains he found in India and the Types X and Y of ANDREWES and INMAN. These last two types had been discarded by BOYD and WHEELER because they were devoid of type specific antigens and

contained group antigens only. In addition to these fourteen types with a single primary antigen, four other types with dual primary antigens are introduced.

The classifications of ANDREWS and INGMAN of BOYD and of WEIL are inserted below for comparison.

TABLE I.  
CLASSIFICATION OF FLEISHER DYSENTERY BACILLI ACCORDING TO DIFFERENT AUTHORS.

WEIL <i>et al.</i>	ANDREWS & INGMAN	BOYD
TYPE	TYPE	TYPE
I	V	Fleisher I
II	W	II
III	Z	III
IV	—	IV (103)
V	—	V (P119)
VI	—	VI (82)
VII	X	—
VIII	Y	—
IX	—	Boyd I (170)
X	—	II (P225)
XI	—	III (D1)
XII	—	D10
XIII	—	P143
XIV	—	P274
I-III	VZ	—
II VII	WX	—
III-IV	—	—
V VII	—	—

All the classifications so far proposed have been based on studies made using agglutinin-absorption tests. In agglutination reactions the antiserum is made to react with the whole antigen exposed on the surface of the bacillus—probably the complete antigenic complex described by BOYD and MESEREAU (1937) and confirmed by MORGAN and PARTRIDGE (1940). However in this phosphorus containing polysaccharide protein complex antigen, the type specificity of the different types of the dysentery bacilli is determined by the carbohydrate component; the protein component is not type-specific (BORNSTEIN 1943).

Different methods for the extraction of this carbohydrate have been used in attempts to diagnose bacillary dysentery (SPANLEY and DAKENFELD 1939) and to classify the organism into their respective types (GONZALEZ and MORALES-OTERO 1942, 1945; DRAFER, 1944). The formamide method of extraction used by GONZALEZ and MORALES-OTERO (1942 and 1945) preserves

the specific carbohydrate of these bacilli and separates it from the other antigenic constituents. The precipitation reaction using this formaldehyde extract is antigen makes possible a rapid and satisfactory method for identification of the organisms. Besides it makes possible the examination of the specific polysaccharide components in these bacilli. Therefore the object of this investigation is to study the present classification in light of reactions given by the specific polysaccharide antigenic fractions and to try to discover the antigenic relationships among the members of the group.

For convenience the nomenclature proposed by Weil *et al* (1944) will be used to designate the different types since it comprises all the races mentioned by all other investigators of this problem. For the sake of completeness we have included strains of *Sh. dysenteriae*, *Sh. allactescens*, *Sh. sonnei*, *Sh. schmitzi* and *Sh. dysenteriae* in addition to the bacilli of the Flexner group.

Type strains of the *Shigella* group were obtained from Dr A. J. WEIL, Dr K. M. WHEELER, The American Type Culture Collection and from the stock of strains isolated in Puerto Rico kept in the School of Tropical Medicine. The type strains used were the following:

TABLE II  
STRAINS USED

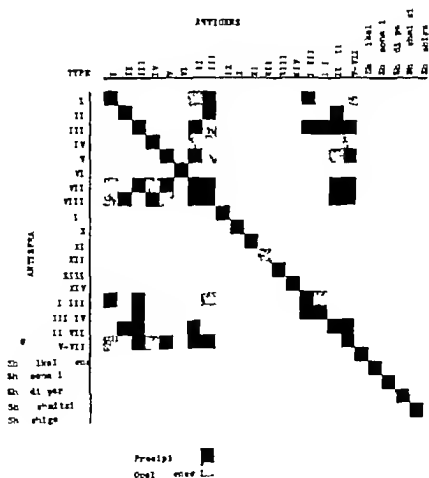
Strain	Type	Source
67-104-V	I (Weil's classification)	Dr A. J. WEIL
63-143-W	II	"
63-143-Z	III	"
66-1-1441	IV	"
63-143-119	V	"
63-125-125	VI	"
66-1-411	VII	"
63-143-3	VIII	"
63-143-170	IX	"
63-143-288	X	"
67-184-D1	XI	"
2763 (J. S. K. Boyd's M270)	XII	"
63-143-143	XIII	Dr K. M. WHEELER
63-143-274	XIV	Dr A. J. WEIL
79-118-3090	I-III	
66-1-570	III-IV	
63-143-X	V-VII	
66-1-1208	II-VII	
9339	<i>Sh. alcalescens</i>	American Type Culture Collection
M Barr	<i>Sh. sonnei</i>	Sch Trop Med San Juan
9755	<i>Sh. dysenteriae</i>	American Type Culture Collection
7018A	<i>Sh. schmitzi</i>	Sch Trop Med San Juan
B-5-7	<i>Sh. dysenteriae</i> (Shiga)	Dr A. J. WEIL



Formolized vaccines were made from each one of the type cultures and antiserum for each one of the strains was prepared in rabbits. Formamide extraction was made from every type strain and precipitation tests were performed with homologous and heterologous sera. The antisera were not absorbed—they were used as obtained from the rabbits. The technique for all these procedures has been described in previous papers (GONZALEZ and MORALEA-OTERO 1942 and 1945).

In Chart 1 inserted below the precipitation reactions for each antigen and antiserum are indicated.

CHART 1



The squares marked in solid black indicate precipitation easily visible to a white hand at the line where the surface of the antiserum and the antigen come in contact. The squares filled with stippling denote visible opalescence at the line of union of antiserum and antigen. Readings were made with the unaided eye, no hand lens was used. Both eyes were considered positive precipitation for interpretation of results.

#### Direct tests

All the type cultures produced antiserum which precipitated with the extract obtained from their homologous strains (see Chart I). Types VI, IX, X, XI, XIII, XIV, XV, *Sh. flexneri*, *Sh. sonnei*, *Sh. dysenteriae* and *Sh. flexneri* (Shiga) formed a precipitate only with their own antiserum. Type VII precipitated its homologous antiserum and the antiserum for *Sh. sonnei*. The extracts from Types I to V gave a precipitate with their homologous serum and with the antiserum for VII or VIII or with both. The Types VII and VIII produced precipitates with their own antiserum and with the sera for Types I to V.

The precipitation reactions of the representative strains of types having dual antigens (Chart I) showed precipitation with more than one antiserum. The polysaccharide extract of Type III precipitated it, Type III, IV and Types I, III and IIIA. The antigen substance of Type III, IV precipitated its own antiserum and the antiserum of Types III and IIIA but did not precipitate the antiserum for Type IV. Besides its homologous antiserum the antiserum prepared for the strains representative of Type II, VII precipitated the antiserum of Types II, III, V, VII and VIII. The other type having a dual antigen, Type V, VII produced precipitation in more antiserum than any of the other dual type strains. In addition to the precipitation produced in its own antiserum it precipitated the antiserum for the following types: Types I, III, IV, V, VII, VIII and II, VII. The precipitation results of this type showed in antigenic pattern the same as that of Type VII.

The agglutination and agglutinin absorption procedures have been used extensively in the study of the antigenic structure of the classification of the *Shigella* group. The immunological specificity of the antigenic complex is due to the carbohydrate fraction and has been used by several authors in attempts to diagnose (SPASSKY and DANIELSSON 1939) and type (GONZALEZ and MORALES OTTARO, 1942, 1945; DANFORTH 1941) the organisms of the dysenteric group using precipitation tests. The carbohydrate constituent of the whole antigenic complex (although obtained denatured by the method of extraction with formamide) can be used for the classification and study of the *Shigella* bacilli by the precipitation reaction which is a very simple and satisfactory test. All the members of the group *Shigella* possess a type specific polysaccharide antigen with the probable exception of Types VII and VIII. Precipitation reaction is revealed by the Type VII and the type with dual antigen

V VII introduced by WEL *et al* showed the same antigenic pattern, suggesting that these two representative laboratory strains belong to the same type.

No cross-reaction was observed between Type XIV (274) and *Sh. alkalescens*. A slight precipitation was produced by the antigenic extract of Type XII (D19) in the antiserum of *Sh. sonnei*; this is a confirmation to cross-reaction between these two types reported by several authors (BOYD 1940; WITZLER, 1944; DRAPER, 1944).

#### CONCLUSIONS.

1 The Types I, II, III, IV V VI, IX X, XI, XII, XIII, XIV of the genus *Shigella* possess a type-specific polysaccharide antigen which characterizes each individual type.

2. Other members of the genus *Shigella* not included in the WEL *et al* schema—*Sh. alkalescens*, *Sh. dysenteriae*, *Sh. sonnei* and *Sh. dysenteriae* (Shiga)—also have a type-specific polysaccharide antigen which characterizes the type.

3 Type VII of WEL's classification (corresponding to Type X of ANDREWS and LEXAN's schema), contains antigenic factors also found in Types I III IV V and VIII.

4 Type VIII of WEL's schema (Type Y of ANDREWS and LEXAN's), contains antigenic factors also found in Types I, II, III, IV V and VII.

5 Types VI, IX, X, XI, XIII, XIV and *Sh. alkalescens*, *Sh. dysenteriae*, *Sh. sonnei* and *Sh. dysenteriae* (Shiga) do not have polysaccharide antigenic factors in common with any other members of the genus *Shigella*. Type XII and *Sh. sonnei* have a common carbohydrate component.

6. Type I-III has two antigenic polysaccharide factors.

Type II-VII has the antigenic factor which characterizes Type II and the antigenic constituents in Type VII.

8 Type V-VII has the polysaccharide specific to Type V and the antigenic components found in Type VII.

9 Type III-IV of WEL *et al*'s schema was found to have only the antigenic factor representative of Type III.

## II. MUTATION OF *SHIGELLA PARADYSENTERIAE* AS REVEALED BY THE PRECIPITATION REACTION

A polysaccharide antigenic component, which determines the specificity of the type, has been demonstrated in all members of the genus *Shigella*. Several investigators (DRAPER, 1944; GONZALEZ and MORALES-OTERO 1945) have used a precipitation reaction for typing the Flexner group of dysentery bacilli. However the formalin method of extraction makes possible the rapid preparation of the polysaccharide antigen, freeing it from other contaminating material that may interfere with the antigen-antibody reaction.

concerned in the precipitation test. The specificity of this precipitation reaction, as demonstrated by the work of GONZALEZ and MORALES-OTERO (1945) offers a very simple and satisfactory means for studying the antigenic structure of the *Shigella* group of bacilli.

The purpose of this study was to examine by the precipitation reaction recently isolated cultures of *Shigella* organisms and cultures that had been kept in stock on tryptose agar (Difco) for the last 3 years or more, and to compare their antigenic structures as shown by this test.

#### EXPERIMENTAL

Antisera were prepared in rabbit against strains of Types I to VIII (WEIL nomenclature, WEIL, BLACK and FARSETTA, 1944) of Flexner bacilli, using the technique described in a previous paper (GONZALEZ and MORALES-OTERO, 1945). The type cultures for the preparation of the vaccines for rabbit immunization were obtained through the generosity of Dr A. J. WEIL. All cultures were studied to ascertain that they had the antigenic polysaccharide component, characteristic of their type, and had undergone no mutation.

Using the technique of FULLER (1938), formamide polysaccharide extract was obtained from recently isolated cultures of Flexner bacilli and from cultures that had been in the laboratory for the past 3 years. These "old cultures" had been kept on tryptose agar (Difco), occasional transplants had been made, whenever necessary, to keep them viable.

Precipitation reaction was performed with the antigen obtained from each culture against antisera for Types I to VIII.

Cultures used belonged to Types I, I to III, II, III, IV, VI and *Sh. sonnei*, the types usually found in Puerto Rico where the organisms were isolated. No organism corresponding to Type V has been isolated in the island. In the following charts, the precipitation analysis of each group is indicated (Squares marked in solid black denote precipitation, the squares filled with stippling indicate visible opalescence).

#### TYPE I (V)

Recently isolated cultures of Type I produced heavy precipitation in the antiserum for Type I (See Chart 1). A slight precipitation was observed in the antisera for VII and VIII but more consistently in the latter. Old cultures of this type gave a marked precipitation in the type serum for I and slight precipitate, or opalescence, with Type III antiserum. Precipitation for antisera VII and VIII was only observed in a few cases.

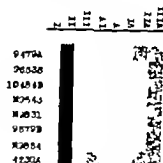
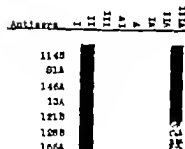
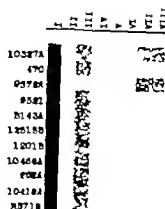
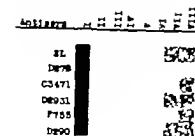
#### TYPE II (W)

The antigenic extract prepared from recently isolated strains of Type II produced marked precipitation in the antisera of Type II and Type VIII (See Chart 1). The precipitate formed in serum for Type II was more than for

CHART 2.  
Type I (V)

Recently isolated cultures.

Old cultures.



VIII In some strains the precipitate for the latter was visible only as an opalescence. The strains of this type of Flexner bacilli, kept for a long time under artificial cultivation, showed no change when reacting with their homologous antiserum, but the precipitation with the Type VIII antiserum was very slight. One of the old cultures gave also a slight precipitation with the antiserum of Type IV.

### TYPE III (Z).

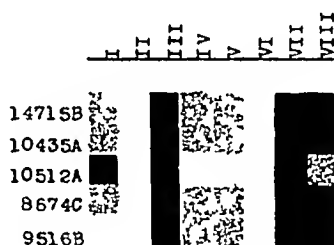
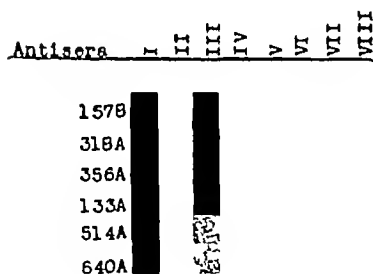
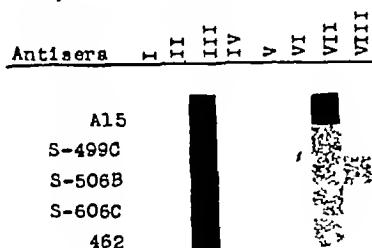
The formamide extract of freshly isolated strains of Type III precipitated Type III antiserum and produced an opalescence in antiserum VII. See Chart 3). A culture, isolated some week before the others, gave a heavier precipitate with antiserum VII. Occasional opalescence was observed in antiserum VIII. In addition to their homologous serum, the strains of Type III, which had been in the laboratory stock for about 3 years precipitated the antiserum for

Type VII strongly. Precipitation was also produced in antiserum V, indicating the presence of a fraction in their antigenic extract for this type. Antiserum VIII was reacted upon by one strain.

CHART 3  
Type III (Z)

Recently isolated cultures

Old cultures



### TYPE I-III (VZ)

Recently isolated cultures of Type I-III formed a marked precipitation in antiserum I and an opalescence in antiserum III (See Chart 2). As the cultures aged, the precipitation in antiserum III became more pronounced. No reaction was observed with any other sera.

"Old cultures" of this dual antigenic type behaved very differently from freshly isolated strains. The precipitation, induced by the antigenic extract in Type I antiserum, appeared now only as an opalescence, while the reaction with antiserum III was very marked in all cases. One culture did not react with antiserum I. The appearance of antigenic fractions, common to Types VII and VIII, was evident, judging by the heavy precipitate formed in these two antisera. A slight reaction was observed for Types IV and V. No precipitation was noticed with antisera II and VI. Although isolated about 3 years before like the other cultures, the strain marked No. 10512 did not show precipitation with antisera IV and V. The reaction with antiserum VIII was

only slight, while the precipitate formed in antiserum I was marked. Apparently the change in this particular strain was not at the same rate as in the other cultures of the same age.

#### TYPE IV (103 BORD).

Type IV showed a greater degree of variation in its antigenic structure than any of the other types. (See Chart 3) Mutation also occurred within a shorter time after isolation. In some instances differences in antigenic structure were noticed a few days after the cultures were obtained from patients. Antigenic extracts of cultures isolated 2 or 3 days prior to the test, produced a marked precipitation in antiserum IV and a slight opalescence in antiserum VIII. One or 2 weeks after isolation the strains began to show a factor found in antiserum V. Precipitation in antiserum VIII was heavy and was also accompanied by marked precipitation in antiserum VII.

Cultures of Type IV which were obtained 3 or more years before the present study exhibited precipitation in all antisera, except that of Type VI. In practically all strains the precipitation with antisera IV, VII and VIII was heavy while with the other sera there was slight opalescence only. Occasionally cultures which also formed a marked precipitate with antiserum V were encountered.

#### TYPE VI (88-NEWCASTLE).

The analysis of the cultures of Type VI did not show any alteration in their antigenic structure due to ageing. (See Chart 4) The organisms of this type precipitated their homologous antiserum only.

#### TYPE *Sh. sonnei*.

Two recently isolated cultures of *Sh. sonnei* were compared with four strains of this type that had been in the laboratory stock for 3 years. The formamide extract of the *Sh. sonnei* organisms produced precipitation in their homologous antiserum only. The antigen of the recently isolated cultures formed a quite noticeable precipitate in the antiserum, but the antigenic extract of the old cultures produced only an opalescence in the serum.

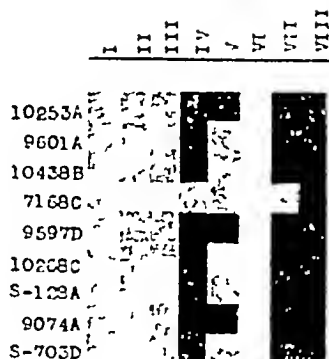
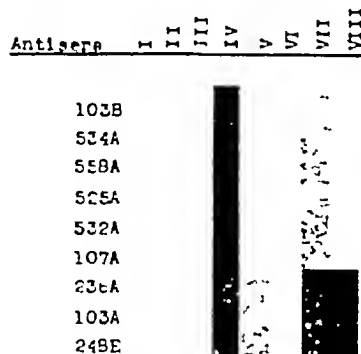
#### DISCUSSION.

Variations in the *Sh. paratyphosae* group have been studied by many investigators (Boyd 1938, TAKITA, 1937, HAMLEY 1937) but none of them has utilized the precipitation reaction in their studies. Their studies of the variations existing in this group of bacilli have been based on the appearance of the colonies and on the alteration of the antigenic structure of the organisms, as measured by agglutination reactions. A comparative study is here presented, utilizing the precipitation reaction as an investigative tool. Theoretically

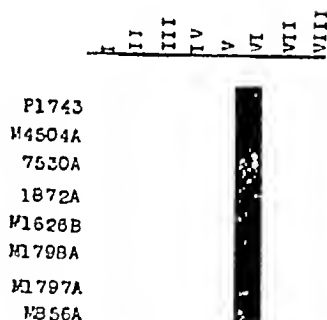
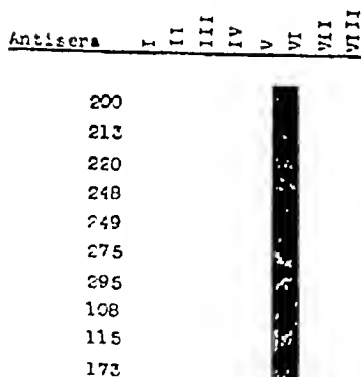
# CHART 4 Type IV (103 Boyd)

Recently isolated cultures

Old cultures



# Type VI (88—Newcastle)



since one of the reagents used for this test is a carbohydrate antigen obtained from the cell of the *Shigella* organism, variations of this carbohydrate component, produced by mutative changes, may become evident in this reaction. In general, the variations that occurred in the different types studied do not come about at any precise time after isolation of the cultures but may appear at various times.

For instance, the changes in the antigenic constitution of Type IV occur sooner than in Type I III, the variation in the latter type becomes evident before alteration in configuration of Types I or II are noticed. Another example of a rapid mutation is the well-known case of *Sh. sonnei*. However, the contrary appears to be the case in Type VI (88—Newcastle), on which artificial cultivation for 3 years did not have any appreciable effect.



The loss of type-specificity in the case of the Flexner bacilli probably proceeds *pari passu* as suggested by Munn (1944) for the capsular polysaccharides of virulent pneumococci and for the Vi somatic polysaccharides of virulent *E. typhosa* and so forth. Loss of the type-specific antigenic carbohydrate factor was observed in *Sh. sonnei* but no other fraction found in other *Shigella* organisms was acquired by this particular type.

The changes in Types I and II were not very significant. As a result of ageing, Type I acquired a fraction common to Type III. There was a tendency for the slight reactions with antisera VII and VIII observed in recently isolated cultures, to disappear as the cultures aged. Although the precipitation was less in all strains studied, Type II retained its reactivity with antiserum VIII. An old strain of Type II precipitated with Type IV antiserum. Both Types I and II retained their type-specific polysaccharide antigen and their study by precipitation did not indicate the acquisition of an antigenic component common to all organisms of the Flexner group—in other words, a group antigen. The variation consisted of a factor found in another type.

From the time of their isolation the cultures of Type III presented a factor common to Type VII. Judging from the precipitation reaction, this antigenic fraction increased as the cultures grew old. On ageing, the organisms of Type III precipitated the antiserum for Type V. Unfortunately it has been impossible to isolate bacilli belonging to Type V in Puerto Rico, consequently this type was not studied in relation to the effects of subculturing on its antigenic structure.

A comparison of the antigenic pattern of the old strains of Type I III and of the standard laboratory strains of Types VII and V VII, as revealed by the precipitation studies, made evident the great similarity in the antigenic mosaic of these three organisms (see Chart 5). In a long series of experiments which he has not published as yet Boro (1940) concludes that Type X is an incomplete variant of Z and not a separate race.

Judging from the pattern of both the mutated strain of Types III and I III and those of standard cultures of Types VII and V VII there exists a great possibility that the X may be a mutant of the VZ subgroup (Type I III) rather than of Type Z (Type III), as believed by Boro. It is significant that the subgroup I III (VZ), when freshly isolated has a greater amount of the type antigen of I though soon after Type III antigen appears and, in old cultures, this latter antigen predominates over the antigen of Type I. As Boro (1938) suggested a recently acquired character occupies a superficial position or is relatively loosely associated with the bacterial body. Consequently the antigenic factor of Type III in subgroup I III is a more primitive and permanent character than factor I in this Flexner type and the former component is more deeply seated in, or more intimately blended with, the body of the bacillus.

The results of the investigation of Type IV (103 Boyd) tend to confirm

CHART 5

## Standard cultures

Antisera	I	II	III	IV	V	VI	VII	VIII
Type VII								
Type V-VII								

## Old cultures

	I	II	III	IV	V	VI	VII	VIII
Type I-III								
14715B								
10435A								
10512A								
8674C								
9516B								

Type III								
M9486								
N L F W								
M9864								
M9493								

Antisera	I	II	III	IV	V	VI	VII	VIII
Type VIII								

	I	II	III	IV	V	VI	VII	VIII
Type IV								
10253A								
9601A								
10438B								
7168C								
9597D								
10268C								
S-128A								
9074A								
S-703D								

Boyd's observations Unquestionably, the antigenic structure of Type VIII (Y) and of old cultures of Type IV are almost identical (see Chart 5) Boyd expressed his belief that 'there is no reasonable doubt that Type 103 is the old Lentz 'Y,' which was not included in its original form in the ANDREWES' series" Our observations have revealed that Type IV, after a variable time in artificial culture, gives rise to variants that are antigenically different from recently isolated cultures of this type Using the agglutination method with his typing sera, Dr WEIL (personal communication) observed that fourteen strains of this type of various periods of isolation, submitted by us, gave numerous cross reactions with antisera I to VIII Using precipitin tests with antigens prepared by an acid extraction, DRAPER (1944) also observed similar cross reactions between the Flexner Type Y and the Flexner Type IV (103 Boyd)

The precipitate formed by antigens of a standard type cultures of VII and VIII and of mutated variants of Types I III and IV in all antisera was not the same quantitatively probably indicating that there is no single group antigen common to all types but a combination of antigenic components, as suggested by the recent work of WHEELER (1944). BORD in his paper stated that the group antigen is more complex in structure than was originally supposed by him, and that it contains several components.

The classification of recently isolated cultures of *Sh. paradysenteriae* by the precipitation method can be performed without great difficulty since the cultures at this stage have not undergone alteration in their antigenic configurations and type-specific antigens predominate. However after they have been subjected to artificial cultivation, the antigenic picture is complicated by the appearance of other components common to types other than the cultured one under classification, therefore the determination of type presents a more complicated situation. Besides providing an easy method of determining the type specific antigen in recently isolated strains, the precipitation reaction presents a very objective means of detecting the secondary components that may have arisen in mutated strains. So far the antigenic pattern of the different mutated strains as revealed by the precipitation reaction are different among races and characteristic of the type. Consequently by precipitation reaction a picture can be obtained which portrays the antigenic architecture of the mutated organisms so that the classification of the strain may be definitely established.

Finally it is suggested that any schema for the classification of the genus *Shigella* should be made from studies of recently isolated strains. Attempts at classification, based on the antigenic analysis of cultures that have been under artificial cultivation for a long time may have its pitfalls, since mutated strains may be mistaken for type-specific representative cultures. As stated above, BORD believed that ANDREWES and ISHAN never had the old Lentz

1 in its original form when they made their classical schema

#### SUMMARY

1 The precipitation reaction affords a simple and satisfactory method for studying existing variations in organisms of the *Sh. paradysenteriae* group.

2 The examination by the precipitation reaction of old strains of Type I III, which have undergone mutation, indicate that they are similar to the standard laboratory Type VII (X). The standard laboratory cultures of Type VII and the type with dual antigen V VII proposed by WILK *et al.* give identical precipitation reactions.

3 Analyses of Type VIII (Y) and old mutated cultures of Type IV reveal them to be similar.

4 Evidence which tends to confirm BORD's observations of mutation in the Flexner group has been presented.

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## SPRUE AND COELIAC DISEASE IN TROPICAL AFRICA

BY

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Tropical sprue is seen mostly in the Far East and affects the European, but it is also met with frequently in the West Indies, especially in Porto Rico. It is frequently said, that dark-skinned races are rarely affected. However, RAMANUJAYYA (1930) affirms that in Southern India sprue is common among Indians. Similarly, MALCOMSON and MURPHY (1931) relate that in their experience sprue in the Indian is an especially common disease. SALAH (1937) describes, from Egypt, what is probably regarded as the first case of sprue in an Egyptian. Non-tropical sprue or idiopathic steatorrhoea is occasionally seen in Europe and many of the patients with the disease give a previous history of coeliac disease, in childhood.

As mentioned above, it is generally held that the dark-skinned races rarely show this condition. Some go as far as to say that it is never seen in the Bantu or Negro. I, personally, have never encountered a case of sprue in an adult African, but there have occasionally been reports of sprue in the adult African from East and South Africa. For instance, LUCKHOFF (1943) found sprue in natives of the Orange Free State. In view of this, perhaps, the assumption of rarity of sprue in the African may have to be revised.

### CASES

I have recently seen a case, which closely resembled coeliac disease in a native infant, aged about 5, in whom the features were typical. It was in a female who was under-sized, the abdomen was protuberant and the liver diminished in size, as determined by percussion. There was the typical wasted appearance of the buttocks, and the stools contained 44 per cent fat, which was split in the normal manner. The stools were loose, frequent and pale. No giardial cysts or amoebic parasites were present. The child was anaemic (hypochromic in type) and, on X-ray, the bones were rarefied. Mentally she was normal. On a high protein low fat diet, together with yeast, liver, iron and vitamins, the child made a slow but excellent recovery.

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\* My thanks are due to Dr B P BERNEY for his criticisms on this paper, and to Dr A P MARTIN, O B E, for his kind permission to publish this paper.

In this case, the general picture and the steatorrhoea were very suggestive of coeliac disease—that is, a disease with a syndrome similar to that of sprue, or as THAYSEN (1929) calls it, the coeliac affection.

I have also encountered a very typical case of coeliac disease in a European male child aged 2 years 7 months.

H was born in Salisbury and enjoyed excellent health until he began to have diarrhoea, which continued for 3 months prior to admission to hospital. The parents observed that the stools were light clay-coloured, bulky and frothy. The child lost considerable amount of weight. When seen in hospital the stools were frothy, pale and had foul odour; they were examined for fat with the following results:—

Neutral ( <i>Le.</i> , unsplit) fat	.. ..	..	Per cent.
Free fatty acids	18.6%	..	5.9
Fatty acids combined as soaps	25.6%	<i>Le.</i> , split fat	44.4
Total fat =			50.3

Percentage of total fat which was split, 83; percentage unsplit, 12.

No parasites were detected in the stools on microscopical examination.

There was moderate degree of anaemia. A blood count showed the following results:—

Total R.B.C.s	3,200,000 per c.mm.
Haemoglobin	.. 63 per cent. (Newcomer)
Colour index	.. .. 0.98
Total W.B.C.s	12,700 per c.mm.
Neutrophils	.. .. 54 per cent.
Lymphocytes	.. .. 33
Monocytes	.. .. 12
Eosinophiles	.. .. 1

The abdomen was distended and the hepatic dullness was diminished. The buttocks were wasted in a typical manner. The child's disposition was irritable, but his mentality normal and alert. An X-ray showed the deficiency pattern of the small intestine. His tongue was not affected. The bones were rarefied.

The stools regained their normal shape, size and fat content after about 2 months. However, at the present time of writing there has been slight relapse with some loss of weight, and diarrhoea.

My own impression of these two cases of coeliac disease was that they were milder in type than the disease as seen in Europe. The same would apply to the adult cases of sprue which will be described now.

In Africa, for some unknown reason, it is said that sprue is rare amongst the European population. Many clinicians are still under the impression that this condition is not seen in the European in tropical Africa. MAXSON BARR (1943) states that the Continent of Africa—especially the tropical region—has afforded very few authentic cases. It is moreover to this authority (1928) that we owe the first description of a case of sprue in a European from Nyasaland.

Amongst troops in North Africa sprue, mostly in mild form and following attacks of dysentery or the administration of the sulphonamide drugs, has recently been described (HOWARD 1944).

Since 1941, I have encountered, in Rhodesia, three European cases, presenting symptoms suggestive of sprue or a sprue-like disease, and similar to the case from Nyasaland, described by MANSON BAHR (1928). Whilst admitting that these cases did not present the classical picture in every detail as seen in the Far East, yet, in most respects, the disease could only be that of a variety of idiopathic steatorrhoea. From the description given of sprue, by MANSON BAHR (1943), I should refer to these cases as incomplete sprue. In none of the three cases were tongue lesions noticed, although in one case the patient did experience a discomfort in the back of his tongue and throat. The absence of tongue changes in the three cases is similar to that found in the case described by MANSON-BAHR (1928) in the European from Nyasaland. It is interesting to note that in many cases of Indian sprue no tongue or buccal changes are seen.

Dysphagia was present to a slight degree in only one patient, who complained of slight difficulty in swallowing associated with rawness of the throat.

In all three cases, diarrhoea was the main symptom—the stools being frequent, pasty in appearance and foul smelling. Their fat content in each case was much elevated. They all contained over 40 per cent fat, which was chiefly split. However, I did not see the typical gaseous or frothy stools so characteristic of classical sprue.

In all, the appetite was very poor, and dyspepsia was common to all the cases. The fractional test meal showed little of note, as in all the hydrochloric acid content was within normal limits. Abdominal distension is said to be marked in typical sprue, but in these cases, there was nothing abnormal to be seen in the external appearance of the abdomen.

Wasting was a common and striking feature. In fact, in each of them, the diagnosis on admission to hospital was that of malignant disease.

In none of the cases was the liver enlarged and in only one was I able to say that its dullness was diminished on percussion. The blood sugar curve, in a typical case, is described as being flat. Unfortunately, I was able to carry out this test in only one case. The fasting blood sugar was 100 mg per cent and the highest maximum level reached was 130 mg per cent, as generally occurs in sprue. The curve itself tended to be flat. A low blood sugar curve—with a rise of 40 mg per cent or less—is very much more frequent in sprue than in normal people (THAYSEN, 1929).

The cholesterol content of the blood is described as being low (MORRIS and MANSON-BAHR, 1926). Again I was only able to estimate it in one case, in which it was 114 mg per cent—a figure well below normal. In the same case the blood calcium estimation, however, was normal—9 mg per cent. The blood calcium need not necessarily be reduced in sprue, although it usually is.

Anaemia was present in all. In one—a female—it was of the hypochromic variety, but in the remaining two, it was macrocytic. The leucocyte count revealed nothing of note in these cases.



The deficiency pattern syndrome as described in sprue and idiopathic steatorrhoea was demonstrated radiologically in one case. It was not sought for in the other two cases. The appearances in the one case were typical. The barium was gathered into small pockets and segmentation was prominent. The normal feathery or herring-bone appearance of the small intestine was absent.

All three cases occurred in people resident in the colony for many years. Two were women, aged 61 and 72, and one a man, aged 57. This is characteristic of the disease which usually attacks middle-aged or elderly people. In all there was a past history of malaria, but in none was there evidence of active malaria: they were probably cases tolerant in the disease. Otherwise there was nothing of significance in any of their previous histories. It is possible that malaria may have been a predisposing factor in the production of the disease (MAXWELL-BAIRD 1943). Sprue is also said to occur in association with amoebiasis or giardiasis, but in none of my cases was either of these diseases found. It might well be that malaria over a long period may have been a percursor in this disease.

Hill diarrhoea is described in India, occurring at heights above 6,000 feet and characterised by dyspepsia, pale, frothy and frequent stools and steatorrhoea, but no anaemia. My cases occurred at heights just below 5,000 feet, but the obvious and severe anaemia present in these cases excluded this diagnosis. All three cases when put on a sprue diet made excellent recoveries and, as far as I am aware, have not relapsed since.

These cases are recorded with the purpose of drawing attention to the occurrence in tropical Africa, of a steatorrhoea which bears the features of sprue in an atypical form: the prognosis being good, once the condition is recognised and the patient correctly treated. It is possible that many more such cases are present, but, perhaps not recognised because of their alleged rarity.

The histories and examinations in the three cases are given in a summarized form. More exhaustive investigation was not possible.

#### CASE 1: Mrs. MAC.

Aged 65. She had been ill for 7 years. She gave previous history of malaria. She was an old resident in the colony having come to Southern Rhodesia many years previously from Great Britain. She was admitted to hospital as a case of malnutrition. The main complaint was weakness, loss of weight and diarrhoea. Her stools were pale and offensive. The fat analysis showed—

		Per cent.
Neutral (i.e. unsplit) fat		3.3
Free fatty acids	13.7%	} Split fat 37.0
Fatty acids combined as soaps	23.3%	
		<hr/>
		Total fat = 40.5
		<hr/>

Percentage of total fat which was split, 91.4, percentage unsplit, 8.6  
 Her blood count showed —

Total R B C s	2,400,000 per c mm
Haemoglobin	76 per cent (Newcomer)
Colour index	1.08
Total W.B C s	4,400 per c mm
Neutrophiles	49 per cent
Lymphocytes	50
Monocytes	1
	"
	"

Anisocytosis marked, polychromasia also of a fairly marked degree. The fractional test meal was normal. With the necessary diet she improved. Her former strength and weight were regained, her stools returned to normal, and she was discharged 10 weeks after admission to hospital feeling exceptionally well.

## CASE 2 Mrs B

European female, aged 61, resident many years in Mashonaland. History went back several years, its main features being marked loss of weight with poor appetite. She was diagnosed as having abdominal cancer and laparotomy was performed in 1942, but no disease was found. In January, 1944, she was admitted into hospital again as a case of inoperable cancer. Her main complaints in hospital were weakness, loss of appetite and weight, and frequent stools which she stated were loose, pale and bad smelling. She had a cachectic appearance. I was unable to demonstrate any growth in the body. An X-ray series of the gastro-intestinal tract revealed no evidence of a tumour. Her lungs were clear. Her blood count was as follows —

Total R B C s	3,820,000 per c mm
Haemoglobin	46 per cent (Newcomer)
Colour index	0.605
Total W B C s	4,200 per c mm
Neutrophiles	48 per cent
Lymphocytes	45
Monocytes	6
Eosinophiles	1
	"
	"

The red blood corpuscles showed a marked degree of anisocytosis and polychromasia. Some poikilocytosis was present but no normoblasts were seen. The fat analysis done on her stools gave the following results —

Neutral (i.e., unsplit) fat	Per cent
Free fatty acids	7.9
Fatty acids combined as soaps	53.3
14.1% } Split fat	
39.2% }	
Total fat	61.2

Percentage of total fat, split, 87, percentage unsplit, 13. There was no occult blood in her stools and the fractional test meal showed a normal acid response.

She was given the usual sprue treatment and made a slow but uninterrupted recovery, being discharged 3 months later feeling very much better. The anaemia and diarrhoea were corrected. Her weight had increased, and just before her discharge from hospital the fat content of her stools was normal.

## CASE 3: Mr. B

Adult European male born in England, aged 58. He lived for many years near Salisbury. He had been ill for 8 months, the chief complaints being diarrhoea, debility with loss of weight and appetite. His stools were loose bulky pale and very offensive but not frothy. Flatulence was marked. He had lost nearly 30 lbs. in weight. He did not complain of pain in his tongue but he noticed there was at times discomfort at the back of it, and occasionally slight dysphagia. From 1910 he had had recurrent attacks of malaria. No previous history of dysentery. He looked very pale on admission to hospital, and his appearance was suggestive of malignant disease.

His blood picture was —

Total R.B.C.s	—	1,300,000 per c.mm.
Hæmoglobin	—	28 per cent.
Colour index		1.00
Total W.B.C.s		2,000 per c.mm.
Neutrophils		84 per cent.
Lymphocytes		33 "
Monocytes		2 "
Eosinophiles		1 "

His stools gave the following findings for fat analysis —

Neutral (i.e. unsplit) fat		Per cent.
Free fatty acids	20.8	4.0
Fatty acids combined as soaps	28.7	47.5
	Total fat	51.5

Percentage of total fat which was split, 81.2 percentage unsplit, 7.8

Wax) of stomach and large bow) were normal. Small intestine showed deficiency pattern syndrome. Occult blood in stools negative. Blood cholesterol = 110 mg per cent. The blood sugar curve showed poor absorption of glucose. The following were the figures: 100 mg per cent. (f.a.) 130 mg per cent. (1 hour), 120 mg per cent. (2 hours), 104 mg per cent. (3 hours). Stools—no pathogenic cysts found.

The patient was put on a fat free high protein diet. Lipid by mouth was given— $\frac{1}{2}$  lb daily lightly cooked. He made a low but remarkable recovery.

His stools became less frequent and after 2½ months in hospital he was passing one normal motion a day. The fat content of the stools had returned to normal and his blood count reached a normal level.

## KWASHIORKOR (INFANTILE PELLAGRA)

Finally a few words with reference to kwashiorkor would not appear to me to be out of place when dealing with either sprue or coeliac disease. This disease is seen throughout most of tropical and sub-tropical Africa, and is chiefly a disease of children between 1½ to about 4 years. I have never seen anything in the adult to resemble this disease either in its onset or course.

Recently I have come to think that these cases were probably not primarily dietetic in origin for the following reason —

1 That in all my cases, the history after careful enquiry, was of a sudden and abrupt onset—in a series of eight consecutive cases it was usually between 2 to 4 weeks only (See Table p 116) It came on suddenly in a child that was perfectly well before, the chief and striking sign being diarrhoea

2 That the mothers all appeared well-covered and they, themselves, showed no signs of deficiency disease Also the other children, if present with the mothers, were perfectly fit, if absent, the mother invariably maintained that they were perfectly fit

3 That the diets in most of the children were not obviously so abnormal as to lead to such a serious disease After careful enquiry most of the children had been given milk, some meat, and vegetables in addition to maize

4 That if kwashiorkor was a nutritional disorder the prognosis should not be so fatal and that some of the infants should recover, especially when seen early, as some of my cases were

I have rarely had a case recover in spite of administering very good and well-balanced diets

My cases were typical in all respects, namely, the skin lesions on the legs, buttocks, perineum, genitalia, and forearms were of crazy pavement type, there was the oedema of the legs (less often of the arms) and face, the wasting, the unhealthy depigmented hairs, the diarrhoea and, at autopsy, the yellow fatty liver

What the cause of infantile pellagra is I do not know I do not believe it to be dietetic Believing that the cause may be a jejunal dysfunction, I did a fat analysis on a small series of cases GILLAN (1934) refers to the sprue-like stools in kwashiorkor, these being often bulky, pale and fatty In one of my cases there was a steatorrhoea of the sprue type, but in another it was slightly raised with poor splitting power, suggestive of either a pancreatic or hepatic dysfunction In the third there was a normal fat response in the stools These results did not support my original supposition that kwashiorkor was an acute sprue-like disease My own belief is that the liver is primarily at fault and that the diarrhoea, skin changes, etc, are secondary manifestations

I have now encountered three native infant cases, who were admitted to hospital with acute diarrhoea of 2 to 4 weeks duration, with slight oedema in one but not in the other two, and at autopsy in each case the typical yellow fatty liver was found No skin lesions were seen Skin lesions are, therefore, it seems, not always present in kwashiorkor However, the fatty liver is apparently an essential feature in all cases

	Case I. Chape (5 years). (Native female.)	Case II. Adams (18 months). (North male infant.)	Case III. Jones (1½ to 2 years).	Case IV Olliver (3 years).
Fat	Total fat 18.7%. Percentage of fat sple 85.0. Percentage of fat mesople 38.0.	Total fat 39.6%. Percentage of fat sple 87.0. Percentage of fat mesople 13.0. Excess fat with normal splitting.	Total fat 20.4%. Percentage of fat sple 23.2. Percentage of mesople 17.8. (Fat high but splitting much below average.)	No fat analysis done.
History	Child quite well until 11 days prior to admission when mother noticed large began to swell. Same day mother noticed rash. Seven days later diarrhoea set in. Typical skin.	Child quite a bit until 2 weeks ago when developed diarrhoea—severe offensive. At same time rash appeared with swelling of face and limbs. Child then taken to which doctor. Typical skin.	Child quite well until one week ago. First noticed swell!! 8 f b d Diarrhoea then started. Typical skin.	DI bow 1½ w ks Typical rash.
Diet	Purridge daily large mug of milk every day more nearly every day	Purridge daily most occasions milk daily—on plain, occasionally tea, no fruit or vegetables.	8 ozes daily most three times a week, green vegetables three times a week.	Diet poor—only mealie meal and porridge. No milk, occasionally meat and vegetables.
Family history	Mother and younger child both look all nourished with smooth skin and well covered.	Mother well nourished.	Mother well nourished.	Mother well nourished.
Autopsy	Yellow liver	Yellow liver	Yellow liver	Yellow liver

	Case V <i>Dill</i> (2½ years) (Female import)	Case VI <i>Takatamba</i> (2½ years)	Case VII <i>Rungo</i> (18 months) (Female import)	Case VIII <i>Kermah</i> (18 months to 2 years) (Female)
Fat	No fat analysis done	No fat analysis done	No fat analysis done	No fat analysis done
History	Quite well until 4 weeks before when developed diarrhoea but only 1 week's duration Typical rash over body, vulva and perineum Crazy pinement skin Hair fine and scanty	Two weeks diarrhoea, quite well before Swollen leg Desquamation of skin 1 week Stools frothy and pale Gametocytes in blood smears	Diarrhoea 3 weeks Yellow pale stools and sometimes greenish Swelling in legs Peeling over trunk and limbs Past history nil	Quite well until 1 month ago developed diarrhoea Stools greenish or yellow at times and frothy Rash appeared later Typical rash and pain
Diet	Milk daily, meat three times a week, *mealie-meal daily occasional vegetables	*Sudzan daily Plenty of vegetables	Sudza, milk, occasional meat and vegetables	Same as Case VII except meat in soup every day
Family history	Mother healthy	Mother healthy	Mother and other three children well	Mother healthy
Autopsy	Yellow liver	Liver greenish yellow	Liver yellowish and enlarged atrophic bowel	Liver yellow Atrophic small bowel

\* Mealie-meal and sudza are maize diets used by natives

## SUMMARY

- 1 Two cases of coeliac disease are described, one in a European and the other in an African child.
- 2 Three cases of (incomplete) sprue are described in adult European
- 3 It is possible that sprue is a not infrequent disease in Africa
- 4 It is suggested that kwashiorkor (infantile pellagra) may not be a nutritional disorder but may be due primarily to acute liver failure. A steatorrhoea may occur in kwashiorkor

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## OVINE PIROPLASMOSES IN IRAQ

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It has been the experience of veterinary surgeons working in the field and of the Laboratory staff to observe an unusual death-rate in sheep in eastern and northern Iraq during the hot months. Many of these deaths were traced to helminthic infestations such as parasitic bronchopneumonia, parasitic gastritis, distomiasis and fascioliasis. Many others were attributed to a condition known locally as "*safra*" or "*zerteg*," which terms denote a yellow discoloration of the carcase. Various factors have been incriminated as producers of *safra*. The presence of several species of plants of the *Senecio* group in many of the pastures in which *safra* prevails suggested the possibility that these deaths may be due to seneciosis. The second possibility taken into consideration was piroplasmosis.

As far as we have been able to ascertain from available literature and deducing from the reports of the Veterinary Department in Iraq, nothing has as yet been published about piroplasmoses in sheep in Iraq, hence it was thought useful to write this paper.

1 During the last week of September, 1943, an outbreak amongst sheep in Baquba (33 km. north-east of Baghdad) was suspected as piroplasmosis by

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the assistant veterinary surgeon in charge. His suspicion was based on the clinical syndrome and the anatomical changes. The symptoms and postmortem lesions observed by him were

"Fever comprising an elevated temperature (105 to 107° F), marked prostration, anorexia, cessation of rumination, accompanied by anaemia, icterus, emaciation and death.

The anatomico-pathological changes consisted of jaundiced mucous membranes, connective and adipose tissues spleen congested and greatly enlarged liver congested marked enlargement of lymphatic glands intestines congested and showing haemorrhagic patches kidneys severely congested and hypertrophied the gall-bladder swollen and distended with dark-green bile.

Blood-smears and smears from liver spleen, lymphatic glands, heart and kidneys were sent to the Veterinary Laboratory in Baghdad for examination. No parasites were detected in smears from the peripheral blood. Very numerous schizonts (Koch's blue bodies), either free, or included in mononuclear cells, were seen in smears taken from lymphatic glands and other organs especially liver and spleen (Fig. 1). The blood picture showed the usual anaemic changes concomitant with theileriasis.

The condition was therefore diagnosed by us as theileriasis in sheep.

Judging by the pathogenicity of the outbreak and the number of animals that succumbed to the infection (estimated at over 20 per cent.) as well as the presence of very numerous plasmospheres in smears from lymphatic glands and haematopoietic organs, we are led to believe that the parasite causative of the disease in question is *Theileria kati* (Dachunkowsky and Urodachovich, 1924).

That the pathogenic agent is *T. kati* and not *T. ovis* is clearly shown by WENTON (1926) and J. GORDON THOMSON and G. NORMAN HALL (1933).

Apart from the economic importance of this disease its geographical distribution makes it worthy of record inasmuch as similar conditions, to our knowledge, have only been reported in Sudanese and Egyptian sheep by MASON (1932) and in Algerian sheep by LESTOQUARD (1923). It is of interest to note, furthermore, that although the majority of outbreaks occur during the hot months cases have also appeared during autumn or spring, as has been our experience.

It is relevant to mention here something about the species of plants belonging to the *Sonchus* group found in Iraq. Two species have so far been identified, viz., *Sonchus oleraceus* and *S. verrucosus*. There are undoubtedly other species that are not identified as yet. It is therefore, not possible to determine to what extent these plants are responsible for causing deaths in animals especially as no experiments have been carried out to establish the toxicological or other effects of these plants. That there are toxic species in Iraq is evident from the various times veterinary surgeons in charge of



2



3



FIG 1—*Theileria hirci* Koch's blue body in smear of liver  
 FIG 2—*Babesia motasi* in peripheral blood  
 FIG 3 *Theileria hirci*—Koch's blue bodies in peripheral blood  
 FIG 4—*Anaplasma ovis* in peripheral blood  
 FIG 5—*Trypanosoma* sp (*T. melophagium* ?)—in peripheral blood

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Fever comprising an elevated temperature (105 to 107° F) marked prostration, anorexia, cessation of rumination, accompanied by anæmia, icterus, emaciation and death.

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Apart from the economic importance of this disease, its geographical distribution makes it worthy of record inasmuch as similar conditions, to our knowledge have only been reported in Sudanese and Egyptian sheep by MASON (1932) and in Algerian sheep by LESTOQUARD (1923). It is of interest to note, furthermore, that although the majority of outbreaks occur during the hot months cases have also appeared during autumn or spring, as has been our experience.

It is relevant to mention here something about the species of plants belonging to the *Sesuvio* group found in Iraq. Two species have so far been identified, viz., *Sesuvio coromandelianum* and *S. verrucosus*. There are undoubtedly other species that are not identified as yet. It is therefore, not possible to determine to what extent these plants are responsible for causing deaths in animals especially as no experiments have been carried out to establish the toxicological or other effects of these plants. That there are toxic species in Iraq is evident from the various times veterinary surgeons in charge of

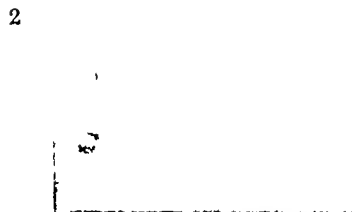


FIG 1—*Theileria hirci* Koch's blue body in smear of liver  
 FIG 2—*Babesia motasi* in peripheral blood  
 FIG 3—*Theileria hirci*—Koch's blue bodies in peripheral blood  
 FIG 4—*Anaplasma ovis* in peripheral blood  
 FIG 5—*Trypanosoma* sp (*T. melophagium* ?)—in peripheral blood



districts, where these plants are known to prevail, are approached by sheep owners for advice regarding deaths amongst sheep which, upon investigation, have not been traced to any bacterial or parasitological causes. This is further corroborated by EVAN GUEST in *Bulletin No 27 of the Department of Agriculture in Iraq*, which we quote verbatim —

"An unidentified plant (almost certainly a species of *Senecio*) has also been reported from many parts of the country as poisonous. It is known on the upper plains and in the foothills of Kurdistan as '*gulikah zar*' (little yellow flower) and in the western desert as '*ward hodhan*', it is probable that these names refer to more than one species. The plant is said to be harmless when dry and can be eaten by animals as fodder, but in the spring, when it is green, it causes a fever in sheep which results in symptoms of giddiness and which may terminate in death. The flesh of animals which have died from this poison is reported to be yellow and discolored."

This yellow discoloration can obviously be interpreted as being the result of cirrhosis of the liver which is characteristic of seneciosis. However, no definite opinion can be formed unless a full survey is made to determine the toxicity of the species prevalent in Iraq. That many species are harmless (*S laevigatus*, *S venosus*, etc.), whereas others are extremely toxic (*S sceleratus*, *S retrorsus*, etc.) is evident from the experiments carried out by various workers in Onderstepoort, South Africa.

2 In May, 1944, a disease diagnosed by the veterinary surgeon in charge as piroplasmiasis, broke out amongst sheep in Mosul District. The symptoms comprised

"Anorexia, cessation of rumination, haemoglobinuria, pyrexia (temperature fluctuating between 104° and 108° F), constipation, accelerated respiration, emaciation and death. Some of the sick animals coughed and showed a nasal discharge."

Postmortem lesions were

"A yellow discoloration of the subcutaneous and abdominal fat, and the fasciae of muscles. Large quantities of a clear serous fluid were seen in the abdominal cavity. This fluid, in some animals, had congealed to form a big, pale-pink, gelatinous clot. The liver was swollen and showed evidence of capillary haemorrhages. The gall-bladder was swollen and distended with greenish-dark bile. The omentum and mesentery were tinged yellow. The kidneys were congested and when incised, the parenchyma was of a greenish-yellow colour. The abdominal lymphatic-glands were also swollen and of a similar colour. The heart was dilated and contained large clots of blood of a vivid, yellow hue. The lungs were congested and showed areas of hepatization and consolidation."

The striking deviation from the characteristic anatomo-pathological picture of this disease was the normal condition of the spleen.

During the same period a similar disease occurred in Erbil District. The clinical and postmortem appearances were identical with those of the Mosul outbreak. One singular feature, worthy of note, although not pathognomonic of the disease, was the presence of epistaxis in the majority of cases in Erbil.

The death rate in both outbreaks was fairly high. Unfortunately no accurate figures of infected sheep or fatal cases can be given by virtue of the nomadic condition of shepherds in this country, who graze their sheep wherever suitable pastures are available, but it has been affirmed to us by the veterinary

surgeons in charge of these two districts that as many as 60 per cent. of the affected animals succumbed to the infection.

Microscopical examination of blood smears and smears from haematopoietic organs of infected sheep dead and alive, proved that the causative factor of the outbreak in the Mosul district was *Babesia motasi* (Fig. 2), whereas sheep in the Erbil district were suffering from a double-infection due to *Babesia motasi* and *Theileria*, as was evident from the presence of plasma bodies (schizonts) in the circulating blood and in smears from internal organs in addition to the parasites (Fig. 3).

Various workers have thus far failed to see the schizonts of *T. lura* (*T. ovis* du Torr 1918) in the peripheral blood, although it was believed *a priori* that they did exist, especially in grave cases, judging by the virulence of the blood. The authors have, however been able to demonstrate definitely the presence of these schizonts in the peripheral blood for the first time.

Sick animals in both areas received 5 c.c., 10 c.c. and 15 c.c. of a 1 per cent. solution of trypan blue intravenously. The injection was repeated in some cases on the 3rd day. Animals in the Mosul District (where babesiosis only was encountered) responded readily to treatment, but no improvement was discerned in the Erbil cases—this may obviously be attributed to the double infection—hence we are of opinion that the organism under discussion is the pathogenic *T. lura*. Besides the chemotherapeutic agent employed, animals were given in both instances magnesium sulphate and sodium chloride in their fodder which consisted of easily digestible food.

3 During the second fortnight of June, 1944 an outbreak occurred amongst sheep in Sulaimaniyah. Judging by the symptoms and postmortem lesions, which are cited hereunder the veterinary surgeon suspected piroplasmosis.

*Symptoms*.—Pyrexia (105° to 107° F.), anorexia, cessation of rumination, progressive anaemia and debility the animals looking extremely cachectic. The mucous membranes were very pallid and the conjunctivae anaemic and showing petechiae. There was nasal discharge of a mucopurulent nature and some of the animals were coughing. Sick animals kept aloof and lay down through prostration until death supervened, and if forced to move they showed marked ataxia.

A postmortem was conducted by the veterinary surgeon in charge on one case only and the lesions noticed were

Pallidity of connective and adipose tissues, an enlargement of the spleen and liver the latter being friable and showing icteric foci. The kidneys were flabby and blanched. The rumen containing solid mass of undigested food. The reticulum, omasum and abomasum were congested. The intestines congested and ecchymosed. The thoracic cavity containing about fifteen ounces of greenish, purulent discharge. Adhesions between the parietal and visceral layers of the pleura were seen, and the lungs showing several abscesses containing pus. The epicardium and myocardium were ecchymosed and studded with petechiae. The bone-marrow looked pale and was of gelatinous nature.

Smears from peripheral blood were positive for *Anaplasma ovis*. The blood picture indicated severe anaemia, with anisocytosis, poikilocytosis and

a comparatively large number of hypochromic macrocytes. The normocytes were indicative of oligochromaemia. The position of the parasites was mostly marginal, but some were sub-marginal and even central. No punctate basophils were seen and normoblasts, macroblasts and microblasts were nil (Fig. 4).

A trypanosome (*Trypanosoma melophagum?*) was also seen in a smear from the peripheral blood of a sheep (Fig. 5).

4 During the month of September, 1944, the advice of one of the writers was sought anent a disease which had killed five out of six sheep in the outskirts of Baghdad. The observations of the owner as regards symptoms were very vague, but anorexia, ataxia, icteric mucous membranes, coffee-coloured urine and marked prostration were noted. The sixth sheep was examined and piroplasmosis was suspected. Blood smears were duly taken and microscopical examination showed a double-infection due to *A. ovis* and *B. motasi*, the latter being very scarce. Before treatment could be adopted the animal succumbed to the disease.

5 Samples of ticks were collected from sheep in the areas in which the outbreaks discussed above occurred, in order to identify them. The specimens of ticks identified were —

(a) *Hyalomma dromedarii*, (Neumann, 1899)

(b) *Rhipicephalus sanguineus* (Latreille, 1804)

(c) *Haemaphysalis annabarinna punctata* (Canestrini and Fanzago, 1877)

No experiments were carried out to establish the role these ticks play in the transmission and propagation of the *Piroplasma* prevalent in Iraq, either in sheep or in other domesticated animals, but that they have been accused and convicted of the transmission of diseases caused by this group of organisms in other parts of the world is evident from the literature published on the subject.

## DISCUSSION

1 Haematozoon parasites of sheep in Iraq received no serious attention previous to the publication of this paper in spite of their scientific importance and pathogenic gravity which is evident from the serious losses frequently incurred.

The parasites encountered during the various outbreaks, and which are recorded for the first time in Iraq, are —

(a) *Theileria lirci* Dschunkowsky and Urodschevich 1924

(b) *Babesia motasi* Wenyon 1926

(c) *Anaplasma ovis* Lestoquard 1924

2 We have noticed that by far the most serious outbreaks of piroplasmosis



occur in eastern and northern Iraq. This may be attributed to the following causes —

(a) During the spring and the hot months most of the sheep in northern and western Iraq owing to extreme heat, scarcity of pastures, shortage of water etc. migrate to the Syrian and the Northern Arabian deserts for grazing purposes, where they obviously escape veterinary supervision. This ostensibly shows that the information available, if any is unreliable and dubious as regards outbreaks of disease whereas in eastern and northern Iraq, where the country is comparatively rich in pastures, shepherds do not have to resort to such measures. It would be appropriate to mention here that sheep from neighbouring countries migrate to eastern and northern Iraq in search of pastures during the spring and the summer and this large conglomeration of sheep along with the mild and favourable weather provides suitable conditions for the multiplication of ticks which are responsible for the transmission of piroplasmosis.

(b) Sheep in eastern and northern Iraq are exposed to severe cold during the winter with hardly any protection against the prevailing gelid and biting wintry wind. This obviously lowers their resistance, especially as the overwhelming majority of flocks depend for their nourishment on what natural fodder they can get which is precarious and practically nil in winter further more, they are subjected to various other parasitic infestations such as peracute gastritis, verminous bronchopneumonia, etc.

3 Ovine theileriosis of pathogenic significance has hitherto been recorded only from Egypt by LITTLEWOOD (1914) and from Algeria by LESTOQUARD (1926) hence this disease was believed to be limited to Mediterranean countries. But now that its existence in Iraq is irrefutably established it is clear that its geographical distribution is not as limited as has been supposed. It should be noted here that Iraq does not import sheep (or goats) from any of the Mediterranean countries, and although the local sheep come in contact with those of neighbouring countries the chances, if they exist, are extremely remote that the infection is contracted from them. This conclusion is based on the fact that the only ovine *Theileria* seen in any of our neighbouring countries, whether situated on the Mediterranean Basin or elsewhere is *T. ovis* Rodhain 1916 (= *T. recondita* Lestoquard 1923) which is non-pathogenic according to LESTOQUARD and LESTOQUARD and EKREMI, q.v.

4 LESTOQUARD claims that the association of two or more infections of piroplasmosis is only seen experimentally after the simultaneous inoculation of several kinds of parasites. He further says that such double or multiple infections are never seen in natural infections, and that should the examination of blood reveal the existence of another haematozoon this should be considered as having already been present in the animal and that the infection was roused during the course of the new attack. He also specified that this only occurs towards the end of the acute attack.

It is not our intention to question this statement as no experimental work was done on this subject, but we nevertheless deem it useful to record our findings in this connection.

(a) In the month of May, 1944, an outbreak of piroplasmosis occurred amongst sheep in Mosul and during the same period a similar outbreak occurred in Erbil. The examination of smears from the blood and haematopoietic organs from sheep in Mosul proved that the disease was due to *B. motasi*, whereas similar smears from sheep in the Erbil district proved a double-infection due to *B. motasi* and *T. hirci*. Since both outbreaks occurred during the same period, and because the two districts are adjacent to each other, one is justified in concluding that the initial attack in both outbreaks was due to *B. motasi*. Should this be the case, how do we account for the presence of *T. hirci* (with Koch's blue bodies) either in the peripheral blood or in smears from the internal organs concerned? If, on the other hand, we were to assume that the initial attack was due to *T. hirci*, basing this supposition on the fact that the animals treated for *B. motasi* did not respond to treatment, to what are we to attribute the presence of *B. motasi* in the peripheral blood in such large numbers? The fact should not be overlooked that smears in both outbreaks were taken when the attack was very acute and in its early stages as evidenced by the havoc wrought amongst the affected flocks and the high rate of mortality.

(b) The other incidence of a naturally occurring double-infection met with is that of anaplasmosis and babesiosis as mentioned previously. We would like to add in this connection that similar double-infections at the onset of acute attacks were also seen in Indo-China by JACOTOT and EVANNO, *q. v.* (1931).

### SUMMARY

- 1 Certain haemosporidia are very pathogenic to sheep in Iraq.
- 2 These are *Theileria hirci* (Dschunkowsky and Urodschewich, 1924), *Babesia motasi* (Wenyon, 1926), and *Anaplasma ovis* (Lestoquard, 1924). These are recorded for the first time in Iraq.
- 3 Instances of naturally occurring double-infections of the blood by these parasites have been met with.
- 4 *Theileria hirci* is not necessarily confined to Mediterranean countries as has hitherto been presumed.
- 5 Schizonts (Koch's blue bodies) of *T. hirci* have been demonstrated in the peripheral blood for the first time.
- 6 A trypanosome (*T. melophagum*?) has also been seen for the first time in Iraq in the peripheral blood of a sheep.
- 7 Samples of ticks collected from infected pastures have been identified as *Rhipicephalus sanguineus* (Latreille, 1804), *Hyalomma dromedarii* (Koch, 1844), and *Haemaphysalis cinnabarinus punctata* (Canestrini and Fanzago, 1877).

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## SEROLOGICAL TESTS FOR SYPHILIS IN TREATED *PLASMODIUM FALCIPARUM* MALARIA

BY

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It is well known that malaria causes false positive results in the serological tests for syphilis in cases where syphilis and yaws can be definitely excluded and that the percentage of such positive results, recorded by different observers, shows great variation

LLOYD and MITRA (1926) expressed the opinion that malaria did not cause false serological results if the technique used was satisfactory

KOLMER (1929) also considered that malaria had no effect on the Wassermann reaction other than anti-complementary action which was common with sera from patients with acute febrile illnesses. However, CUMMING (1935), in an intensive investigation carried out by the U S Public Health Service, submitted sera from non-syphilitic malarial patients to four laboratories for Wassermann and Kahn tests. KOLMER reported from his laboratory 19.4 per cent positives on the sera submitted to him.

The most important recent contribution to the subject has been made by KITCHIN (1939), who made a systematic study of the Wassermann and Kahn tests before, during and after naturally induced malaria in twenty-five non-syphilitic subjects, and found that

(1) Positive reactions were obtained in all cases where malaria developed clinically. In two cases the Kahn was negative and Wassermann positive, and in two others the reverse held.

(2) The majority were positive 3 or 4 weeks following inoculation.

(3) In a few instances the first positive test was found before, and in a few instances after, the period of clinical activity.

(4) The optimum time for the highest percentage of positives was the period 15 to 21 days from the last previous paroxysm.

(5) *P. vivax* infections tend to induce a greater number of false positives than *P. falciparum* infections.

(6) The serological tests were negative before and after the positive results, showing that the results were due to malaria and not in any way due to syphilis.

(7) Two patients gave positive results in tests 4 or 5 days before the development of the clinical attack.

In a malarial hyperendemic area it is essential to have some facts regarding the frequency of false positive results in cases of treated malaria and how to distinguish such false positive results from specific reactions due to syphilis. It is obvious that, in such an area, the responsibility of the serologist in the interpretation of the results is increased. He must avoid attaching the stigma of the diagnosis of syphilis to a patient showing a false positive serological test for syphilis due to malaria and he must not miss a latent syphilis with a positive serological result obtained during the course of an attack of clinical malaria. This is particularly important with patients suffering from non-syphilitic venereal disease (gonorrhoea, soft sore and lymphogranuloma).

inguinale) who also have malaria. This is not an uncommon occurrence as exposure to the former infection implies some relaxation of personal anti-malarial precautions in a mosquito infested environment. It is not unusual for cases of gonorrhoea and soft sore in malarious areas to develop attacks of clinical malaria while under treatment for the venereal disease. One such case—an acute gonorrhoeal urethritis treated with sulphathiazole—developed malaria with a positive Kahn reaction. The question of a diagnosis of concomitant or latent syphilis was aroused but as all subsequent blood tests were negative the reaction was shown to be due to the malarial infection.

This investigation was carried out at R.A.F. Hospital, Takoradi, Gold Coast, in 1943 on European Service personnel with acute *Plasmodium falciparum* malaria and positive blood slides. All cases when diagnosed were put on routine quinine and mepacrine therapy which consisted of quinine grains 10 t.d.s. for 48 hours combined with mepacrine 0.1 gramme t.d.s. for a further 7 days. The cases investigated were as far as possible, consecutive admissions without selection and all grades of clinical severity were included. Syphilis was excluded in all cases by documentary evidence, history, clinical examination and follow-up of the cases showing positive serological tests.

#### METHODS AND RESULTS

Blood was withdrawn within 24 hours of the finding of the malarial parasites in the peripheral blood and again on the 10th day from the initial paroxysm.

The sera were tested in parallel against the Ide, Meinicke and Kahn antigens using standard methods.

Positively reacting sera were subjected to a Kahn verification test by the differential temperature technique (KAHN 1940).

The results are given in the following tables —

TABLE I  
SERA EXAMINED ONset OF MALARIAL ATTACK

Total 100	Ide.	Meinicke.	Kahn.
Positive		4	2
Doubtful	1	0	2

TABLE II  
SERA EXAMINED ON 10TH DAY FROM ONSET

Total 100	Ide	Meinicke.	Kahn.
Positive	2	4	4
Doubtful	0	1	1

TABLE III  
TESTS PERFORMED AT ONSET AND REPEATED ON 10TH DAY ON THE SAME PATIENT  
NUMBER OF PATIENTS EXAMINED—50

(a) Positive			(b) Doubtful		
	Onset	10th day		Onset	10th day
Ide	2	1	Ide	1	0
Meinicke	3	3	Meinicke	0	1
Kahn	2	3	Kahn	1	1

TABLE IV  
TOTAL TESTS CARRIES OUT IN MALARIA—200

	Ide	Meinicke	Kahn
Positive	4	8	6
Doubtful	1	1	3
% positive	2	4	3

### DISCUSSION

In treated *P. falciparum* malaria false positive results to the serological tests for syphilis using three flocculation techniques were found in approximately 3 per cent of the patients examined. The results obtained by the three methods were, considering their varying sensitivity, in satisfactorily close agreement (Ide 2 per cent, Meinicke 4 per cent, Kahn 3 per cent.)

A comparable investigation was carried out in West Africa by ELMES and FINDLAY (1944) on a similar group of some eighty patients. These authors found twenty-three false positive results with the Kahn test and four with the Ide. In West Africa I found the Ide test to be a very useful slide test for syphilis with a fairly satisfactory correlation with the standard Kahn reaction. The following table shows the results obtained in West Africa on 1,000 sera tested against the Ide, Kahn and Meinicke antigens in parallel.

In primary and treated syphilis the Meinicke reaction was the most sensitive test and the Ide the least. In a few cases of secondary syphilis examined, the results were in entire agreement. No non specific reactions with any of the three antigens were obtained on testing the sera from cases of gonorrhoeal and non specific urethritis, "soft sore," lymphogranuloma inguinale, etc.

Discrepant results however were obtained on examining the sera from a group of 200 healthy adult Africans for evidence of latent yaws or syphilis.

TABLE V  
1 000 SERA—153 REACTING SERA.

	Positive.		Doubtful		Total reacting.
	Number	%	Number	%	
Ide	73	48.6	1	15.7	99
Meinicke	123	80.4	7	17.8	150
Kahn	100	65.4	28	18.3	128

Some of these sera gave weak reactions and a number showed no reaction with the Ide antigen but weakly flocculated the Kahn or Meinicke antigens or both. In this group the percentage of positive results with the three antigens was as follows—

Ide 28 per cent. positive    Kahn 37 per cent. positive    Meinicke 48.5 per cent. positive.

The most sensitive flocculation test under tropical conditions appears to be the Meinicke and this sensitivity is not apparently associated with any loss of specificity. Yet even using this very sensitive antigen only 4 per cent. positive results were obtained with sera from cases of treated *P. falciparum* malaria.

False positive results were obtained in these cases of treated malaria during the first 14 days. One case was doubtful when tested within 24 hours of the clinical onset and was negative at 10 days and in another case the reverse held. The sera were negative in the cases personally followed up, 1 month after discharge i.e. about 40 days from the first paroxysm. In untreated malaria KIRCHEN (1939) found that the highest percentage of false positives was obtained between the 15th and 21st days and the result remained positive up to 2 months. It would appear that the results obtained in treated and untreated cases show a considerable variation.

Adequate control of the malaria with efficient treatment has a marked effect on the false positive results in malaria. Untreated cases all develop positive results (KIRCHEN 1939). Positive results found early in the disease sometimes show serological reversal during the first 10 days of treatment (DANIEL 1943) while no spontaneous reversal occurs during the early stages in untreated cases. The optimum time for obtaining false positive result is later in untreated than in treated malaria and the seropositivity persists longer in untreated cases.

There are, at present in use, two methods of distinguishing false positive results due to malaria from true syphilitic reactions—serological follow-up and eradication tests.

Malarial false positives do not persist long after the period of clinical

activity—1 month in treated cases and 2 months in untreated ones. Serological follow-up for 2 months after discharge from hospital following malarial treatment, should allow of a differential diagnosis.

Kahn verification tests by the differential heat method were performed on positively reacting sera but the technique failed to confirm the non-specific nature of the reaction in malaria. The inherent technical difficulties in carrying out this test satisfactorily in the tropics precludes its use as a satisfactory verification test.

It is considered that the RICHARDSON potentiation test is a better verification test for syphilis, yet even this method gives equivocal results with positively reacting sera from malarial patients and does not always confirm their non-specific nature. One is therefore drawn to the inevitable conclusion that verification tests cannot at present be relied upon to distinguish the reactions in malaria from true syphilitic reactions and reliance must be placed on serological follow-up.

If there is careful control to exclude syphilis and yaws in the cases being investigated, it has come to be recognized that malaria causes fewer false positives with modern flocculation methods than was at first assumed. Any investigation carried out in a yaws endemic area on coloured patients is without significance because of the frequency of positive results to the serological tests for syphilis given by healthy adults.

Even though syphilis and yaws are excluded in the cases under investigation there are other factors which affect the results and which, if not fully appreciated, tend to confuse the picture.

The following have been shown to be of importance

- (1) *The strain of infecting parasite*  
*P. vivax* gives a higher number of false positives than *P. falciparum* (KITCHEN, 1939)

- (2) *The clinical stage of the disease*  
 False positive results have been reported during the incubation period of malaria (KITCHEN, 1939)

In the acute stage of treated malaria the highest number of false positives is obtained in the first 10 days and the seropositivity does not persist after 1 month.

False positive results have been recorded in sub-clinical malaria by DAWBER, 1943, and KITCHEN, 1939, but the cases subsequently developed frank clinical malaria. All work tends to show that the false positive results are related to the clinical activity of the disease. If sub-clinical malaria were capable of causing false positive results one would expect to see such positive results in personnel in a malarial hyperendemic area who are on mepacrine.



suppressive therapy. This has not been my experience and it would appear that positive results obtained in sub-clinical (or chronic) malaria should be viewed with suspicion.

### (3) Antimalarial therapy

False positive serological tests due to malaria are affected by antimalarial treatment.

(a) The number of false positive results obtained are lower in treated than untreated cases.

(b) False positive results are obtained most frequently in the first 2 weeks in treated and during the 3rd week in untreated malaria.

(c) The period of post-clinical seropositivity is reduced by treatment.

### (4) The serological tests for syphilis used.

The results obtained on the same sera show great variation with the serological test employed (BURNLEY, MATS and ICKRANT 1940) even when allowance for individual variation in interpretation is excluded. On the whole the Wassermann is more sensitive to false positive sera in *P. falciparum* malaria and the Kahn in *P. vivax*.

## SUMMARY

1. Serological tests for syphilis have been carried out on 200 patients under treatment for *Plasmodium falciparum* malaria.

2. Three flocculation tests were employed in parallel—Ide Kahn and Meincke—and the false positive results obtained were approximately 3 per cent.

3. Methods for distinguishing such false positives from specific syphilitic reactions are discussed.

4. Factors, apart from syphilis and yaws, which affect the results are mentioned.

## CONCLUSIONS

In treated *Plasmodium falciparum* malaria fewer false positive results in the serological tests for syphilis are obtained than in untreated malaria.

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## TECHNIQUE

*Calibration of Instrument*

A stock standard solution containing 100 mg miracil hydrochloride in 1 litre 0.1 N HCl is prepared. This is stable for at least 3 months, if kept in the dark. Two ml of this solution are diluted to 50 ml with distilled water. Each 0.5 ml of this dilute solution, when added to 5 ml of fresh oxalated blood, produces a miracil content equivalent to 40  $\mu\text{g}$ /100 ml. A series of 5 ml blood samples can, therefore, easily be prepared containing total amounts of miracil hydrochloride equivalent to concentrations of 40, 80, 120, 160 and 200  $\mu\text{g}$ /100 ml respectively.

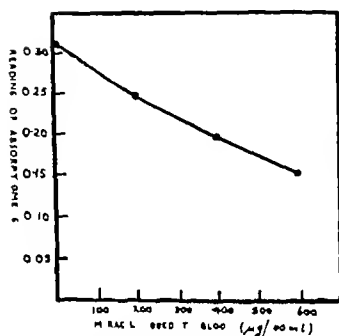


FIG 1—Results of dye-laking procedure using samples of blood containing known concentrations of drug  
*Spekker absorptiometer, Ilford spectrum red filter*

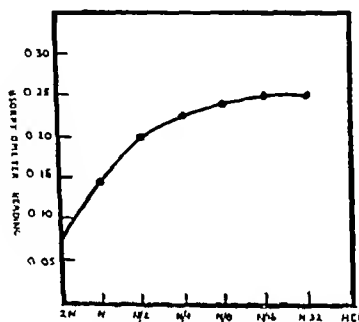


FIG 2—Relation of intensity of yellow colour of miracil solutions to the concentration of HCl present  
*Spekker absorptiometer, mercury light, Ilford spectrum violet filter*

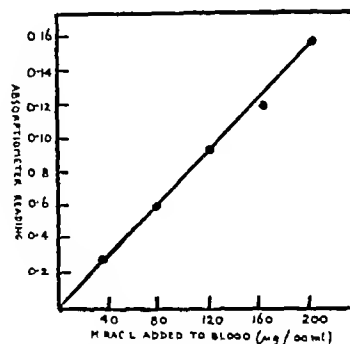


FIG 3—Yellow colour method for miracil. Results obtained from samples of blood containing known concentrations of miracil  
*Spekker absorptiometer, mercury light, Ilford spectrum violet filter*

Each sample is haemolyzed with 10 ml distilled water, and then made alkaline with 2.5 ml N NaOH solution. It is best to carry out the whole of this procedure, from the preparation of the 5 ml blood sample onwards, in a 500 ml separating funnel. The clear brown solution thus formed is treated with 0.1 ml caprylic alcohol, and thoroughly extracted by shaking with 25 ml of ether. It is essential to shake the separating funnel at least 200 times. The emulsion thus formed is as a rule easily broken by the addition of 2 ml of acetone and is allowed to stand for at least  $\frac{1}{2}$  hour until the two layers are well defined. If the emulsion proves especially obstinate, a few drops of absolute alcohol may be added as a further aid to inducing adequate separation. After the lower layer has been run off, the ether layer is collected and set aside in a stoppered bottle while the lower layer is returned to the funnel and again extracted with a further 25 ml ether. Separation at this stage

## PRINCIPLE

Miracil is extracted from laked blood in the presence of sodium phosphate into ethylene dichloride. To this solution in ethylene dichloride is a bromo-thymol blue (buffered at pH 7) and the mixture is shaken, with formation of a compound of miracil and bromo-thymol blue which is soluble in ethylene dichloride. Since the uncoupled dye is insoluble in the organic solvent, a partition of the dye takes place, and the amount of dye thus removed from the aqueous phase is measured.

## TECHNIQUE

*Bromo-thymol blue reagent*—This is prepared in two stages follows—

First stock solution is made by dissolving 40 mg. of solid dye in 100 ml. of 95 per cent alcohol. Secondly 4 ml. of this solution are diluted to 100 ml. with buffer containing 3.63 grammes of  $\text{KH}_2\text{PO}_4$  and 14.35 grammes of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  to the litre.

To 5 ml. of fresh whole blood, laked with 10 ml. of water are added 3 ml. of 0.1N  $\text{Na}_2\text{HPO}_4$  and then 30 ml. of ethylene dichloride. The mixture is shaken vigorously for 5 minutes in a mechanical shaker and then centrifuged for 15 minutes at 2,500 revolutions per minute. The upper aqueous layer is separated away leaving the organic layer covered with a pellicle of protein material.

The ethylene dichloride is transferred to another bottle, the pellicle being left behind and is then washed once with 60 ml. of 10 per cent  $\text{NaOH}$  and twice with the same volume of water. At each stage the mixtures are mechanically shaken and later separated by centrifuging as in the original extraction. 25 ml. of the ethylene dichloride layer removed by means of a graduated pipette, and placed in a small stoppered measuring cylinder. 2.5 ml. of bromothymol blue reagent are then added and the mixture is shaken mechanically for 15 minutes to bring about coupling between miracil and the dye, and is centrifuged.

One ml. of the upper aqueous layer is removed and treated with 10 drops of 10N  $\text{NaOH}$ . The resulting blue colour is read in a Spekker absorptionmeter using matched cups, and the original miracil content is determined from a graph previously prepared. The graph is drawn from the readings obtained, after treating by the above method a series of samples of normal fresh blood to which miracil has been added in known amounts. A graph derived in this way is shown in Fig. 1 and it will be seen that the amount of miracil removed from the aqueous solution (as indicated by diminution in intensity of its colour) is proportional to the concentration of miracil present.

## YELLOW COLOUR METHOD

Miracil exhibits a strong yellow colour when dissolved in hydrochloric acid and it was found that this colour varied with the concentration of miracil present showing an analogous though opposite tendency to that of the fluorescence of mepacrine (BROOKS and UNDERHILL 1943). This variation in the case of miracil is shown in Fig. 2.

By transferring the miracil from 5 ml. of blood into 1 ml. of 0.04N  $\text{HCl}$  and reading the colour of the latter in a Spekker absorptionmeter using matched cups, blood concentrations of the order of 10 to 100 g./100 ml. could be handled.

## TECHNIQUE

*Calibration of Instrument*

A stock standard solution containing 100 mg miracil hydrochloride in 1 litre 0.1 N HCl is prepared. This is stable for at least 3 months, if kept in the dark. Two ml of this solution are diluted to 50 ml with distilled water. Each 0.5 ml of this dilute solution, when added to 5 ml of fresh oxalated blood, produces a miracil content equivalent to 40  $\mu\text{g}/100\text{ ml}$ . A series of 5 ml blood samples can, therefore, easily be prepared containing total amounts of miracil hydrochloride equivalent to concentrations of 40, 80, 120, 160 and 200  $\mu\text{g}/100\text{ ml}$  respectively.

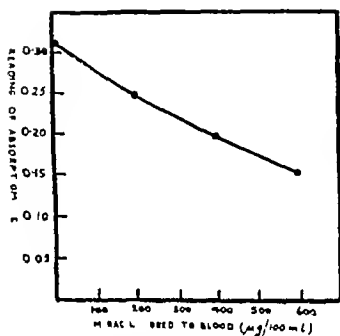


FIG 1—Results of dye-laking procedure using samples of blood containing known concentrations of drug  
*Spekker absorptiometer, Ilford spectrum red filter*

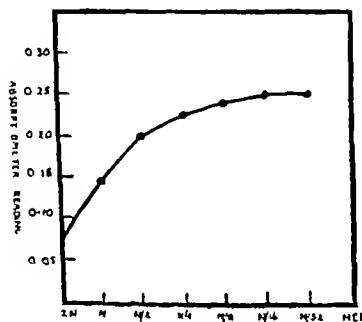


FIG 2—Relation of intensity of yellow colour of miracil solutions to the concentration of HCl present  
*Spekker absorptiometer, mercury light, Ilford spectrum violet filter*

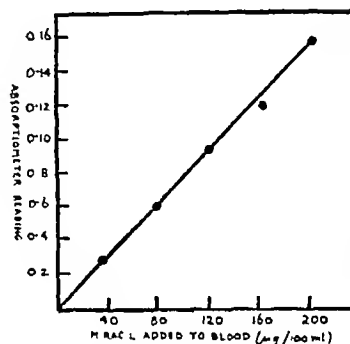


FIG 3—Yellow colour method for miracil. Results obtained from samples of blood containing known concentrations of miracil  
*Spekker absorptiometer, mercury light, Ilford spectrum violet filter*

Each sample is haemolyzed with 10 ml distilled water, and then made alkaline with 2.5 ml N NaOH solution. It is best to carry out the whole of this procedure, from the preparation of the 5 ml blood sample onwards, in a 500 ml separating funnel. The clear brown solution thus formed is treated with 0.1 ml caprylic alcohol, and thoroughly extracted by shaking with 25 ml of ether. It is essential to shake the separating funnel at least 200 times. The emulsion thus formed is as a rule easily broken by the addition of 2 ml of acetone and is allowed to stand for at least  $\frac{1}{2}$  hour until the two layers are well defined. If the emulsion proves especially obstinate, a few drops of absolute alcohol may be added as a further aid to inducing adequate separation. After the lower layer has been run off the ether layer is collected and set aside in a stoppered bottle while the lower layer is returned to the funnel and again extracted with a further 25 ml ether. Separation at this stage

usually takes place quite easily. Occasionally however it may be necessary to add a few drops of absolute alcohol. The ethereal phase from the second shaking is added to that obtained from the first, the residual aqueous fluid being discarded. The separating funnel is now washed out with 20 ml. 0.5  $\times$  NaOH solution to remove any scum adhering to inner surface, and the combined ether extracts are returned to the cleaned funnel, and shaken well with a further 20 ml. 0.5  $\times$  NaOH. The alkali layer is run off, and the ether extract washed twice by shaking well with distilled water whose reaction has been adjusted to pH 7.2. Each of these procedures is liable to be followed by some degree of emulsion formation, but standing for 10 minutes is usually sufficient to bring about an adequate degree of separation. It has been demonstrated that the small amount of ether contained in the emulsions, after this period of time, is usually negligible, and its loss does not materially affect the result.

The ether extract after the final washing, is freed as far as possible from the lower aqueous layer and transferred to a clean dry 100 ml separating funnel. This transfer is carried out by pouring the ether through the neck of the larger funnel, and not by running it off through the stem so as to avoid carrying over any traces of emulsion which might dilute the acid to be added. 1 ml. of 0.04  $\times$  HCl is now introduced and the small funnel thoroughly shaken by hand for several minutes. There must be at least 600 to-and-fro movements.

After being allowed to settle the clear lower layer which is now as a rule, perceptibly yellow in colour is run off into a micro-cup (volume 0.5 ml.) and its coloration measured in a Spekker photoelectric absorptiometer using an ultra violet light source and spectral violet filters (Mford 601).

A typical calibration curve is shown in Fig 3. It can be seen that there is a satisfactory straight line relationship between the final extinction values, and the known concentration of miracil hydrochloride. The results have been found to be consistently reproducible, but it is advisable to check the curve at intervals to allow for possible variations in the instrument.

#### DETERMINATION OF MIRACIL CONCENTRATION IN BLOOD.

5 ml. of oxalated blood are treated as already described, and the miracil content determined (in terms of miracil hydrochloride g./100 ml.) from the calibration curve.

#### SOLUTIONS REQUIRED

*Stock standard miracil hydrochloride solution* 100 mg miracil hydrochloride are dissolved in 0.1  $\times$  HCl and made up to 1,000 ml by the addition of more 0.1  $\times$  HCl. This keeps for 3 months.

*Diluted miracil standard* 2 ml. of the above stock standard are made up to 50 ml. with distilled water.

0.04 N-HCl is prepared by diluting 2 ml N-HCl to 50 ml with distilled water, which has been exactly adjusted to pH 7.2 by the addition of dilute NaOH or HCl.

It is of extreme importance that the two final washings of the ether extract be carried out with water, which has been adjusted exactly to pH 7.2. This adjustment was carried out in our experiments with a Lovibond comparator using phenol red as indicator, and adding dilute HCl or NaOH as required. For values in the neighbourhood of 20  $\mu\text{g}$  or less of miracid solidus 100 ml blood, it is preferable to use 10 ml of oxalated blood instead of 5 ml.

## RESULTS

### (a) Recoveries of added miracid

It was found that, using a calibration curve derived in the manner described above, and then checking the results against given amounts of miracid added to a series of blood samples, satisfactory recoveries could be demonstrated. The results of a set of such trials are shown in Table I.

### (b) With blood from animals after dosage with miracid

Our experiments with animals were limited in scope, being confined to demonstrating that our chemical procedure would be applicable to the range

TABLE I  
RECOVERIES OF MIRACID ADDED TO BLOOD IN THE LABORATORY

Amount added to 5 ml blood in $\mu\text{g}$	Equivalent level $\mu\text{g}$ per 100 ml	Amount recovered in $\mu\text{g}$ Figures in brackets are percentage recoveries		
		First experiment	Second experiment	Third experiment
2	40	1.8 (90)	1.6 (80)	2.2 (110)
4	80	3.5 (87)	4.1 (102)	3.5 (87)
6	120	5.8 (96)	6.6 (110)	6.6 (110)
8	160	7.6 (95)	7.3 (91)	7.6 (95)
10	200	9.7 (97)	10.0 (100)	10.3 (103)

of concentrations which might reasonably be expected in animals receiving therapeutic doses of miracil. It was first shown that blood from untreated rats, rabbits and monkeys (as well as human subjects) gave a negligible reading when subjected to analysis. Results obtained from rabbits and rats receiving miracil by parenteral injections are set out in Tables II and III.

TABLE II.  
ALTES FOR RABBIT BLOOD AFTER INTRAMUSCULAR DOSAGE.

Dose	Time after dose in hours.	Blood miracil in $\mu\text{g}$ per 100 ml.
50 mg. per kg. body weight	1	125
	2	125
	3	118
	Died in 6 hours	—
.5 mg. per kg. body weight	2	45
	20	.5
	45	15

TABLE III  
VALUES FOR RABBIT BLOOD FOLLOWING INTRAPERITONEAL DOSAGE  
(A and B are different series  $\frac{1}{2}$ )

Dose.	Time after dose in hours.	Blood miracil $\mu\text{g}$ per 100 ml.	
		A	B
.5 mg per kg body weight.	2	40	—
	4	80	40
	20	—	30

Blood was obtained from the rabbits by venipuncture and from the rats by ventricular puncture.

#### COLORIMETRIC METHOD FOR URINE.

A procedure based on the same principles as that described for blood can be applied equally well to urine. Since the absolute amount of miracil

that can be conveniently recovered from this source is much greater than is the case with blood samples, a stronger solution of hydrochloric acid can be used for the final reading of the colour and direct comparison with a standard solution of miracil can be employed in lieu of reference to a calibration curve.

The technique which we have found satisfactory for the analysis of urine samples is as follows —

To 50 ml of urine in a separating funnel is added a sufficient volume (2 to 3 ml) of 0.3 N NaOH to render the mixture alkaline to litmus and also 50 ml of diethyl ether, the funnel is vigorously shaken. After settling, the lower layer is collected in a flask and the upper layer transferred to a stoppered bottle. The lower layer (which is likely to contain some emulsion) is now returned to the funnel and shaken with a further 50 ml of ether. On separation, the upper layer is added to the ether extract obtained from the first operation while the lower is again returned to the funnel and mixed with about 100 ml of water. This tends to break up any remaining emulsion enabling a further amount of ether extract to be recovered. The whole of the ether extract is now washed with 10 ml of 0.3 N NaOH any emulsification being counteracted with a few drops of absolute alcohol. The ether layer after a further washing with water, is then shaken with exactly 10 ml of N-HCl. The acid layer is withdrawn and its colour compared with standards made up by dissolving 5 and 25 mg respectively of miracil in 1 litre of N-HCl.

If the reading indicates a urinary content of less than 0.5 mg/l of miracil, it is preferable to repeat the procedure using 100 ml, or even 200 ml, of urine.

### RESULTS

When miracil was added artificially to normal urine in the laboratory the amount recovered over a range corresponding to 1 to 5 mg/litre did not differ from that added by more than 10 per cent.

In the case of rabbits receiving a parenteral dose of 25 mg miracil per kg body weight, the urine may be expected to contain about 2 mg per litre.

### DISCUSSION

The colorimetric method described has the virtue of great simplicity. By running a series of estimations concurrently, manipulating one while another is standing, a large number of estimations can be carried out in a relatively short time. It is important that the larger separating funnel used for the initial separation should be scrupulously clean, since any adherent particles on its wall seem to encourage the formation of troublesome emulsions. Using fresh samples of blood we have never found a blank value greater than the equivalent of 5  $\mu$ g per 100 ml, but if blood is kept at room temperature for longer than 2 hours, readings corresponding to 15  $\mu$ g may be reached. The question of the volatility of the solvents is one which merits some mention.



since the drug under consideration is intended for combating a tropical disease, so that field trial are likely to be made in countries where the atmospheric temperatures are much higher than in the United Kingdom. It seems to us that since the initial experimental work with miracil is likely to be done in a temperate climate and that there is a prospect of air-conditioning plants being installed on an increasing scale in research laboratories in tropical countries, it was justifiable to introduce a procedure involving an ether-extraction. Trials with the substitution of high-boiling petroleum ether were not successful, nor were attempts to utilize amyl alcohol and amyl acetate. On the other hand, it was found that extraction with ethylene dichloride, as in the dye-laking method, followed by re-extraction of the ethylene dichloride with 85 per cent. lactic acid offered a possible, though more tedious, alternative colorimetric procedure.

Regarding the figures in Tables II and III, it should be mentioned that the animals whose blood we tested had received only single doses of the drug; it is likely that during therapeutic régimes involving repeated doses the levels encountered will approximate to the earlier and higher ones in our Tables.

So far there are not sufficient data available to enable any definite statement to be made as to the specificity of either of the methods described but we consider that the colorimetric is probably the more specific as well as the more sensitive. Support for this opinion is found in its negligible blank value and also in the fact (first noticed when using the method on rabbits urine) that some metabolic derivative of miracil I produced by animals receiving the drug which differs from the drug itself in its behaviour towards the extractants. Thus, although this derivative is extracted from alkalinized urine by ether it does not pass from the ether into hydrochloric acid and therefore does not enter into the final colorimetric measurement. Presumably a similar substance may exist in blood but again it would be prevented from interfering with the colorimetric reading by being eliminated during the extraction process.

### SUMMARY

1. A method of determining the concentration of miracil in blood by extracting it and measuring its yellow colour is described.
2. A preliminary account of a dye-laking method is also given.
3. The application of the colorimetric procedure to urine is described.
4. Some results obtained with these procedures are recorded.

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# AN EPIDEMIC OF LOUSE-BORNE RELAPSING FEVER IN KENYA

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## INTRODUCTION

This paper deals with what is probably the first epidemic of louse-borne relapsing fever to occur in East Africa. Buxton (1939) has stated that except in a belt stretching from the Sudan to Senegal and in Abyssinia, this form of the disease is absent from Tropical Africa. In the past, relapsing fever in Kenya has always been tick-borne, with the possible exception of a small outbreak amongst Abyssinian refugees in the Northern Province, which Cormack (1937) assumed on epidemiological grounds was of the louse-borne variety. During the last 10 years there have been 3,236 cases of the tick-borne

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\* We have to thank the DIRECTOR OF MEDICAL SERVICES for permission to publish this paper.

Special acknowledgement is made to Dr C R. PHILIP for his great help in initiating the control measures, his unique knowledge of the Kenya coast and its tribes was invaluable in obtaining the confidence of the people.

We take this opportunity of thanking the African assistants and Messrs J P McMAHON, J O HARPER, K P BAILEY, and A E C HARVEY, of the staff of the Division of Insect-Borne Diseases, Medical Research Laboratory, for their steady work in the field and in the laboratory. We are indebted to Major LOWBURY and Dr DOWDESWELL for performing the agglutination tests.

disease with eighty-two deaths. The origin of the recent louse-borne epidemic in Kenya appears to have been Arabia, unfortunately little is known of relapsing fever in that country and the exact strain concerned is apparently undetermined, though Hirst (1931) refers to the occurrence of tick-borne anemias in Southern Arabia.

According to CHOCOLAN (1940), devastating epidemic of louse-borne relapsing fever began in Upper Guinea after the first world war and spread across the Sudan killing at least 100 000 people. Towards the end of the second world war serious outbreak occurred in French North Africa, which is stated by STUART (1945) to have affected nearly 40 000 people. When, late in 1945 an epidemic situation was recognized in Kenya, it was realized that similar development was threatened here and control measures were at once instigated. At the same time the opportunity was taken of investigating the type of *Spirochaeta* involved, with special reference to its effect on animals and its behaviour in arthropods.

## HISTORY OF THE EPIDEMIC

The disease was introduced into Kenya from Seihut, South Arabia, in February 1945 when a number of Arab dhows arrived in Mombasa with several cases of relapsing fever on board. As most of the passengers on the dhows were heavily infested with lice and no ticks could be found it was assumed from the beginning that the fever was louse-borne. The infected dhows were put into quarantine and the sick were isolated on shore.

Shortly afterwards a few cases began to appear in Mombasa and on the mainland in the vicinity of the island. The disease finally spread into the native reserve, the first case occurring in the reserve at the end of July 1945; no cases, however, were diagnosed until August. The majority were jaundiced and were at first thought to be cases of yellow fever. The disease reached the native reserve in the following way. A Mombasa resident, suffering from relapsing fever, was visited by two women from Marakani. The women returned home after staying a week. 4 days later they fell ill. Subsequently the rest of their family and various relatives and friends also contracted the disease and fourteen out of eighteen died.

During October the disease assumed epidemic proportions, and by the beginning of November the three local hospitals were unable to cope adequately with the influx of patients.

By the time we arrived in the district the disease had been prevalent for several weeks and was well recognized by the majority of the local inhabitants. Careful questioning of chiefs and elders could therefore be relied on to give a fairly accurate picture of the situation.

It was discovered that the epidemic had spread as far as Vitengeu to the north, Ndavaya to the south and Taru in the west (see Fig. 1). The population of this area was approximately 110 000. There were about 1,550 cases with 390 deaths. The mortality in untreated cases was 40 per cent. Women were infected more frequently than men or children and the relative proportions were: Men, 28 per cent. Women, 47 per cent. Children, 25 per cent.

There was a higher mortality among the men than the women which appeared to be due to the high proportion of old men affected; the latter were not only more lousy but had less resistance to the disease.

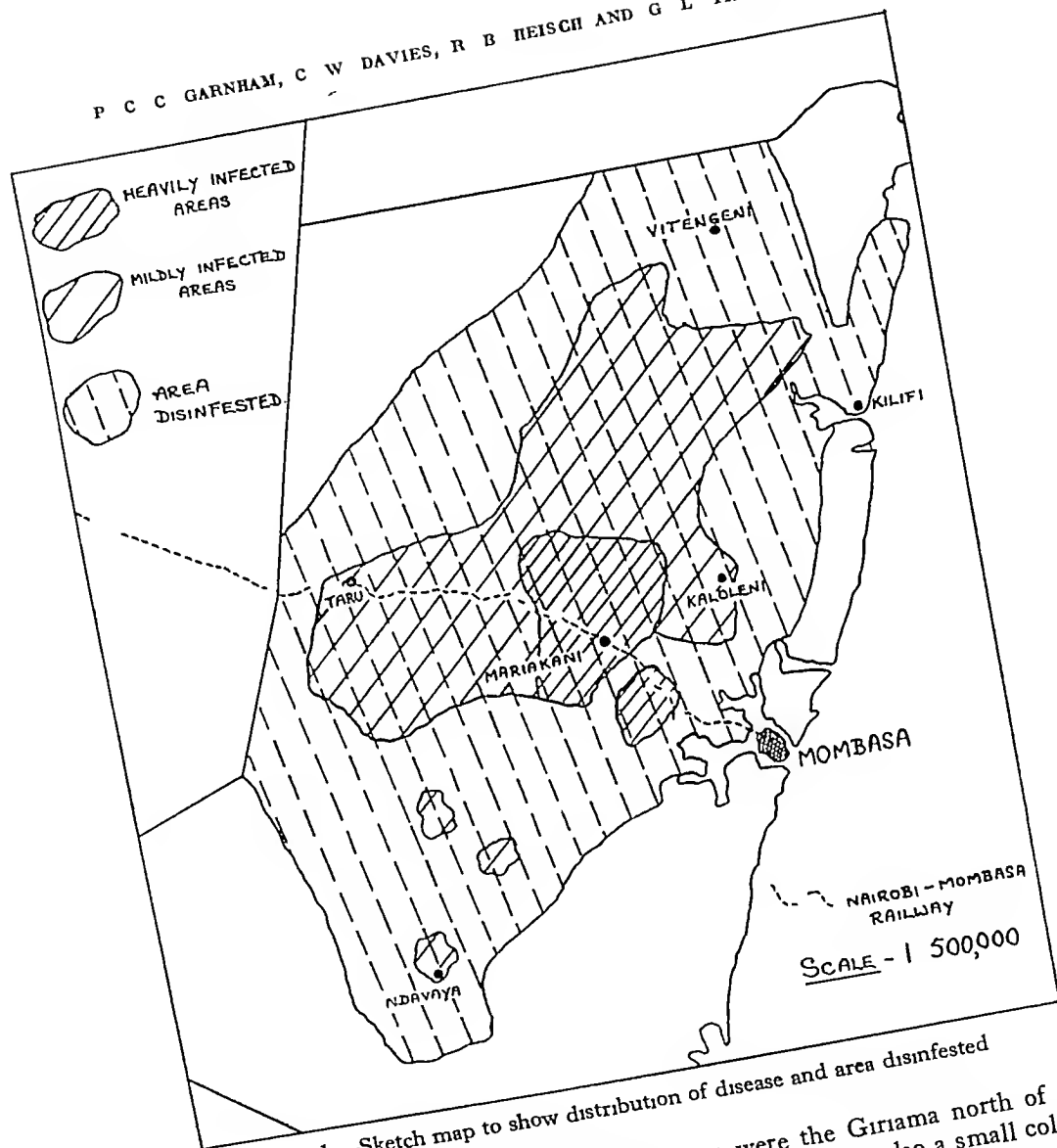


FIG 1—Sketch map to show distribution of disease and area disinfested

The principal tribes in the epidemic area were the Giriama north of the railway line and the Duruma to the south of it. There was also a small colony of a few thousand Wakamba, all of whom escaped infection.

The disease appeared to spread along the trade routes and undoubtedly trading between markets played a considerable part in the dissemination. Motor lorries and the railway probably affected both the speed and the distance of the spread. Another significant factor was the native funeral, which lasts for several days and is attended by all relatives and friends. The women sleep

in the same house as the corpse and the men under the trees outside. It was found that many of the people who were present subsequently developed the disease, the women being affected in particular.

Conditions of malnutrition prevailed in the area and it is possible that these were an important factor in the epidemic, for as pointed out by CHUVO and WAI (1938) epidemics of house-borne relapsing fever are usually associated with famine.

Further spread of the disease was prevented by control measures which are now described.

### CONTROL MEASURES.

The first measure taken was to relieve the overcrowding in the local hospitals. A temporary hospital was opened at Mariakani in the most heavily infected area and the accommodation at Kaloleni hospital was increased by the erection of tents. The following preliminary measures were also put into effect —

1. Rapid removal of cases to hospital by ambulance.
2. Destruction of lice by boiling clothes.
3. Suspension of the elaborate funeral ceremony.
4. Discouragement of travel, or when essential, disinfection of travellers at railway stations and bus termini.
5. Disinfection in houses from which new cases were reported.

The success of these measures depended on the co-operation of the people. There still exist in this part of the country ancient indigenous organizations (*kayas*) which even today have considerable influence. Members of these *kayas* gave us their full support, and it is perhaps significant that south of the railway line, where the *kaya* is more powerful than in the north, all funeral ceremonies ceased almost immediately and the disease was extinct by 10th January 1948 whereas a few cases still continued north of the line.

The destruction of lice by boiling clothes was carried out successfully in the greater part of the infected area in spite of the great lack of water due to drought.

Though every effort was made to discourage travel, facilities were provided at hospitals and dispensaries whereby travellers could be protected by having their clothing impregnated with DDT dusting powder.

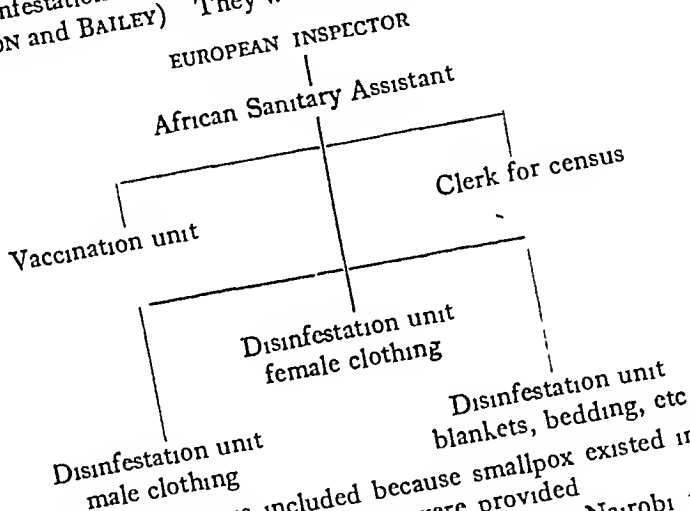
A disinfecting unit accompanied the ambulance whenever it went out to collect a new case and the bodies, clothes and bedding of the friends and relatives were powdered with DDT. The importance of this "case-contact delousing service" was stressed by CRAWFORD-BENSON (1948) in reference to the Naples typhus epidemic.

Disinfection was carried out by special teams at the principal railway stations and bus termini. All those wishing to travel were disinfested and

provided with a certificate which was valid for a week. The railway authorities and bus drivers refused to issue a ticket to any person without a certificate. All these measures were maintained throughout the campaign except for the boiling of clothes which ceased when mass disinfection began.

### THE DISINFESTATION CAMPAIGN

Three disinfection teams were formed each led by a European (Messrs CARTER, WILSON and BAILEY). They were constituted as follows —



(A vaccination unit was included because smallpox existed in the district at the time). Lorry transport and tents were provided. Two teams worked north of the main Mombasa-Nairobi road and one south. It was found that each team could treat approximately 500 persons in an hour. Before disinfecting the inhabitants of any particular area, a previously selected centre was visited and a time arranged with the local people. On arrival the team converted existing buildings into disinfecting rooms. If there were no suitable buildings, *bomas* of hessian and poles (Fig 2) were erected. The people were put in queues as they arrived and with the help of local police were made to pass through the *bomas*. When inside, the people removed their clothes and were powdered with DDT, particular attention being paid to the hair and waist-line. The clothing was dealt with outside by being placed on a mat and showered with powder which was well rubbed in. Coats, trousers and dresses were turned inside out, special attention being given to the *marinda* (frilled skirt). (See Figs 3 and 4). For applying the powder various types of powder-gun were tried, but their output was insufficient and they invariably clogged up after a few minutes. Tins with perforated lids were used instead and as long as the holes in the lids



FIG. 2. The portable disinfection house with bags of dusting powder and the mats on which the clothing was treated.

FIG. 3.—A red *marseide* (which photographs black) being treated. It is spread out as much as possible while being sprinkled with DDT powder.

FIG. 4.—The same *marseide* as in Fig. 3. Rubbing in the powder. The cloth now photographs almost white, showing the thoroughness of the powdering.

FIG. 5.—A group of women wearing *marseide*.

FIG. 6.—A single *marseide* spread out. It may comprise 40 yards of material. When worn, this material is gathered up to about 1 yard in length and folded over a string which is tied round the waist.

large enough, they were found to be excellent They produced clouds of powder and could be rapidly refilled

NATURE AND EFFICACY OF THE DUSTING POWDER  
WEBSTER (1946) recommends as an anti-lice powder a 10 per cent mixture of DDT in China clay, ground in a suitable ball mill Unfortunately, local supplies of DDT were limited and a 5 per cent mixture was all that could be prepared The base was finely powdered kaolin This mixture adhered to the body and clothes, a considerable quantity remaining even when the clothing was beaten

The efficacy of the powder was tested as follows —

(a) Lice were removed from several *marinda* immediately after dusting Out of forty-six lice collected, thirty-four were dead after 20 hours and of the twelve still alive, nine fed readily After 48 hours, nine more were dead, leaving three alive This test was rather exacting for obviously the dust could not penetrate immediately into all the pleats of the skirts though later contact between lice and DDT was almost certain

(b) Numbers of *marinda* were searched 48 hours after treatment and all were found free from lice

(c) Fifty-six *marinda* were examined after 10 days, four contained from one to three lice It was ascertained that the four infested skirts had been washed soon after disinfection, subsequently, people at disinfecting centres were warned not to wash their clothes The other fifty-two skirts were louse-free and still showed plenty of DDT powder

Using official census figures, it was calculated that 90 per cent of the population in the infected area had been disinfested by the end of the campaign Allowing for the people absent from the district at the time of the safari, it is estimated that nearly 100 per cent of those actually living in the area were treated

Fig 7, page 148, shows the number of cases admitted to Mariakani, Kilifi and Kaloleni (C M S) hospitals for weeks ending 24th November, 1945 to 19th January, 1946 It will be observed that the graph falls steeply in two places, the first drop was presumably caused by the boiling of clothes and the second by the mass disinfection

By the end of the campaign, cases were occurring in negligible numbers and the epidemic was considered to be over

## CLINICAL FEATURES, PATHOLOGY AND TREATMENT

The following observations were made on patients in the temporary relapsing fever hospital at Mariakani

The onset of the disease was sudden, with high fever, shivering, severe headache and aching in the muscles and joints The disease was severe and



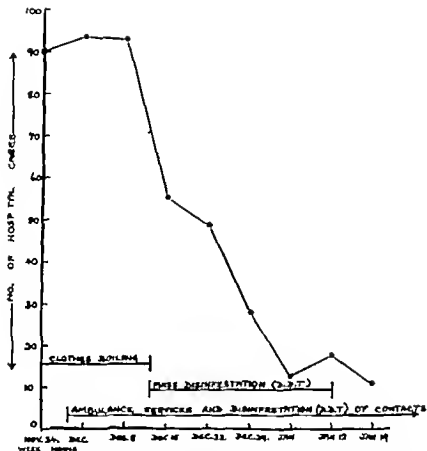


FIG. 7.—Graph showing hospital admissions in relation to the control measures.

all patients looked very ill in the early stages many were unable to walk and others were extremely ataxic.

The temperature rose rapidly to 103° or 104° F and remained elevated for 5 to 8 days in 64 per cent. of the patients 2 to 4 days in 18 per cent. and 7 days in 18 per cent. The temperature was usually remittent during this period although high continuous fever was sometimes seen. The initial pyrexial period ended with a fall in temperature by crisis in 67 per cent. and lysis in 33 per cent. The subsequent apyrexial interval was only truly apyrexial in 45 per cent. the others showed a low remittent or intermittent fever which gradually fell by lysis or continued until the first relapse.

The different types of temperature curve may be classified as follows —

- (1) High remittent pyrexia, fall by crisis or lysis apyrexial interval.

- (2) High continued pyrexia, fall by crisis or lysis, apyrexial interval
  - (3) Intermittent pyrexia, fall by crisis or lysis, apyrexial interval
  - (4) High remittent, continued or intermittent pyrexia, fall by crisis or lysis, interval of low remittent or intermittent pyrexia
  - (5) Any of the above forms followed by one or more relapses
- Temperature curves (1), (2) and (3) are similar to those recorded by CHUNG and CHANG (1939) in Chinese louse-borne relapsing fever. The temperature curve most frequently seen in the present epidemic showed a high remittent fever during the pyrexial period followed by an interval of apyrexia or low intermittent pyrexia.

Thirty-two per cent of the patients relapsed, usually once but sometimes twice. No patient had more than two relapses. Most of the relapses occurred between the 11th and 16th days of the disease, although a few began on the 10th day. Relapses in monkeys infected with the same strain of spirochaete showed a more constant periodicity and occurred between the 10th and 12th days of the disease. The relapses were usually less severe than the original attack and their duration was shorter, 2 days in 68 per cent of the cases, 1 day in 21 per cent, and 3 days in 11 per cent. The peak temperature was usually lower than that of the initial attack ( $100^{\circ}$  to  $101^{\circ}$  in 47 per cent,  $104^{\circ}$  and over in 30 per cent and  $102^{\circ}$  to  $103^{\circ}$  in 23 per cent). Only a few second relapses were seen and these were rather indefinite in character, one, however, was clear-cut and occurred exactly 8 days after the onset of the first relapse. The relapses often ended by lysis whereas fall by crisis was more usual for the initial pyrexia.

Nearly all the patients complained of severe headache when their temperatures were raised. Frontal headache was common, sometimes accompanied by aching in the temples and occipital region. Meningism, with negative Kernig's sign, was observed in several cases. Delirium was not infrequent and some of the patients showed great mental excitement. The mental symptoms often persisted for several weeks after the temperature became normal.

Joint and muscle pains occurred in approximately 63 per cent of the cases. Aching and tenderness in the calf muscles was intense in many of the patients. The thigh muscles were sometimes affected but to a lesser degree. Backache was usually severe. The knees and ankles were the joints most frequently and severely affected. The shoulders, hips and elbows were sometimes also involved. A few patients complained of pains in the chest which appeared to be due to fibrositis.

The pupils of fifteen out of thirty-three patients examined on admission either did not react to light or reacted sluggishly. The accommodation reflex was present. These signs resemble the Argyll Robertson phenomenon, but the pupils were usually dilated and the normal light reflex returned later in the course of the disease. The knee and ankle jerks were absent or sluggish in half the patients.

examined at the onset. In one patient the reflexes were much exaggerated, with bilateral ankle clonus. Slow improvement occurred after the symptoms had persisted for several weeks. Forty "healthy" natives were examined as a control and thirteen had sluggish knee and ankle jerks. This gives a figure of 30 per cent. which does not differ widely from that obtained for the sick persons. In some patients, however knee and ankle jerks previously sluggish or absent became normal and even brisk later in the disease, which suggests that relapsing fever may have played a part in their aetiology. The diminished reflexes in the tribesmen were perhaps due to some widespread vitamin deficiency.

The abdominal reflexes were absent in about 33 per cent. of the cases they usually returned later in the disease.

The gait was often very unsteady especially during the pyrexial periods. On attempts to walk the patient's legs tended to cross over each other as in the scissors gait. While standing there was often considerable difficulty in lifting up the legs (as in "marking time"). Rombergism was absent. The ataxia appeared to be due to severe muscular weakness.

Only a few cerebrospinal fluids were examined and these were clear showing no increase in cells but a monkey injected with cerebrospinal fluid from a relapsing fever patient subsequently developed the disease. The specimen of fluid was not contaminated with blood and presumably contained spirochaetes though they were not found on dark ground examination. Transmission by cerebrospinal fluid has been obtained by several observers, and CHUNG (1933) succeeded in infecting splenectomized squirrels with cerebrospinal fluid from patients suffering from the Chinese strain of louse-borne relapsing fever.

Vomiting is usually marked in louse-borne relapsing fever but it was only observed once in the recent epidemic. ROGERS (1939) found that vomiting occurred in 30 to 60 per cent. of all louse-borne relapsing fever cases, and according to STRONG (1945) vomiting is frequent during the febrile periods and is often bilious in character. Diarrhoea was present in 28 per cent. of the cases but was never severe.

The liver was enlarged and tender in 68 per cent. of the cases. It was often difficult to palpate the liver because of muscular rigidity over the hypochondrium the enlargement which was never marked could often be measured only by percussion. Tenderness over the liver was usually extreme. CHARTERS (1942) observed a similar tenderness and rigidity over the liver in relapsing fever patients in Abyssinia. 28 per cent. of the patients were jaundiced.

The spleen was enlarged and usually tender in 75 per cent. of cases. The enlargement was seldom more than 2 fingers. It is probable that some of the splenic enlargement was due to malaria.

Cough was present in 34 per cent. and appeared to be caused by a tracheitis. Bronchitis was uncommon and rhonchi were seldom heard on auscultation.

The heart was enlarged in sixteen of thirty-three patients examined in the early stages of the disease. The apex beat was felt 1 to 2 inches to the left of the nipple line and the percussion note was dull  $\frac{1}{2}$  to 1 inch to the right of the sternum. The cardiac enlargement had usually disappeared by convalescence and it was presumably due to dilation. Pulmonary and mitral murmurs were present in ten of the series.

Three pregnant women with relapsing fever aborted after being admitted to hospital, and information received from other parts of the epidemic area indicated that pregnancy was usually terminated by the disease. There was no evidence to prove the congenital transmission of relapsing fever and no spirochaetes were found in the organs or placentas of two foetuses from infected patients. These findings were similar to those recorded by GARNHAM (1936), who found no spirochaetes in the placenta of an infant who developed relapsing fever 9 days after birth.

The blood was usually examined for spirochaetes during the febrile periods. Thick films were stained by Giemsa or Field's stain and thin films with Leishman. In 237 cases, all of which were probably relapsing fever, only 65 per cent showed spirochaetes. Blood examined during the initial pyrexial periods generally contained many spirochaetes whereas during relapses they were absent or very scanty. A number of fresh blood preparations were examined by dark ground illumination, this showed the spirochaetes very clearly and enabled a rapid diagnosis to be made.

Total white cell counts were done on a small series during the febrile periods. Only a few had a leucocytosis and this was slight in degree (10,000 c mm to 15,000 per c mm). Several of the white counts showed a relative increase in the monocytes.

The Kahn reaction was positive in only three of thirty-six specimens of blood examined at different stages of the disease, the three positives were probably due to syphilis or yaws. These findings differ from those of KORSHUM and LIEBFRIED (quoted by STRONG, 1945), who also took blood at different stages of the disease and found 56 per cent positive for the Wassermann reaction.

The sera of 38 out of 40 patients agglutinated the Kingsbury strain of *B. proteus* in titres varying between 1/40 and 1/2,000. Agglutination was absent or slight for the OX2 and OX19 strains. The test was repeated after a week in ten of the cases and showed a marked rise of titre in six of them, two cases rose to 1/1,250. ROBINSON (1942) obtained similar results while investigating Abyssinian relapsing fever and found that many sera agglutinated "OXK" in a high titre.

The Van den Berg reaction was negative in five out of ten patients, equivocal in three, and positive (indirect) in two. This meagre evidence, although of little value, suggests a haemolytic type of jaundice.

The mortality rate is discussed on page 142, it was 40 per cent in untreated cases and 4 per cent in those treated in hospital.

## TREATMENT

Adults were treated with novarsenobillon intravenously (0.45 to 0.6 gramme) and children with acetylarman intramuscularly (0.02 gramme). The drugs were given while the temperature was rising. Spirochaetes remained in the blood up to 8 hours after an injection of N.A.B.

Although the course of the disease often appeared unaffected by treatment, the low mortality in the treated cases, as compared with the untreated, suggested that the drug had a beneficial effect. WOLMAN (1944) found that intravenous arsenic had no effect on the course of Abyssinian relapsing fever. (It may be of interest to mention here that acetylarman caused the blood of two monkeys heavily infected with *S. duttoni* to become negative within 12 hours. Untreated monkeys almost invariably died when infected with the *duttoni* strain.)

## PATHOLOGY

There was little human material available for study. One elderly African male was admitted to hospital moribund. Spirochaetes were found in his blood and he was given N.A.B. He died 12 hours later and a postmortem was done 2 hours after death. All the tissues were deeply jaundiced. There were no haemorrhages and no macroscopic changes which we could regard as specific. No spirochaetes were found by dark-ground examination of films from the various organs. The histological changes were not specific and no spirochaetes were seen in Levaditi-stained sections. Several months later a child died of relapsing fever presumably tick-borne, in Nairobi and at postmortem examination 6 hours later the findings, with one exception which is referred to below were similar.

Diagnostic changes were described by RUSSELL (1932) consisting of areas of cellular infiltration in and around the Malpighian corpuscles. These were found in sections of the spleens of our monkeys infected either with *S. recurrentis* or *duttoni*. We observed no differences in the appearance in the two infections. We were unable to confirm RUSSELL's observation that spirochaetes could be found in silver stained spleen sections when they were invisible in films. They were numerous in sections of spleens from animals in which the blood contained spirochaetes at death, but were not seen in those in which the blood had become negative. One spleen from a monkey which died after a first relapse showed considerable and apparently recent diffuse and trabecular fibrosis.

In the livers of the monkeys and of the two human cases the Kupffer cells were swollen. The liver cells showed focal degenerative changes.

One of the monkeys with *duttoni* infection developed ataxia before death and sections of its cerebellum showed nuclear degeneration, chromatolysis and vacuolation of the cytoplasm in the ganglion cells. Similar changes were present in the cerebellum of the child and in that of another monkey in which there were no apparent nervous symptoms. They were not found in other parts of the brain.

Meningovascular inflammation is recorded in relapsing fever (SCOTT, 1944) but was not observed in this investigation

#### THE LOUSE AS THE VECTOR OF THE DISEASE

On epidemiological grounds, it was suspected from the beginning that the louse was the insect vector concerned. The disease was occurring in epidemic form and the history of the outbreak indicated a probable origin in louse-infected immigrants from Arabia. The much higher incidence in women than in men suggested that the louse-infested pleated skirts (*marinda*) of the women had an aetiological significance (Figs 5 and 6, page 146).

It has previously been stated that the colony of Wakamba natives, living in the midst of the Wadaruma tribe, were never affected by the disease. Wakamba women do not wear *marinda* and the tribe as a whole is much cleaner in its habits. It was also noted that amongst males it was largely the aged who contracted the disease—the old men were much more lousy.

Louse counts of *marinda* showed a low infestation, viz, an average of four per woman, in only one case were many lice (300) recovered. A possible explanation of the small numbers is that the boiling of clothes had begun 2 or 3 days before the counts were made.

In order to confirm the theory of the louse transmission of the disease, the following experiments were made—

Lice (*Pediculus humanus corporis* Degeer) were collected from patients who had spirochaetes in their blood and were treated as follows—

- (1) They were crushed on slides and stained with Leishman or Gordon's stain
- (2) Emulsions were made and examined by dark ground illumination
- (3) Lice were ground in water or normal saline and the emulsion was inoculated into animals. None of the animals became infected, their susceptibility to the disease was confirmed by injection of known positive material 3 or 4 weeks later.

No spirochaetes were discovered in this series, presumably because the lice were still in the negative phase or were uninfected. Probably all these insects had made their first infective feed less than 7 to 10 days before examination or inoculation.

In the next experiments, the lice were kept alive for periods up to 13 days before examination and spirochaetes were then easily demonstrated. Table I (page 154) gives the results. The lice were collected from a case of relapsing fever on the 12th December, 1945, and were divided into two batches. The first batch was fed on a "clean" monkey and spirochaetes were first seen in crushed lice on the 17th December. Transmission by inoculation (on 17th and 19th) into monkeys (M9 and M12) was successful. The second batch was fed twice daily for 5 successive days on positive cases of the disease, they were afterwards kept alive by feeding on ourselves for a further period of

13 days. Very numerous spirochaetes (Fig 8) were seen in crushed lice from this batch on the 21st December and again on the 24th December on which day another monkey (AL8) was successfully infected.

During the experiments the lice were kept in an incubator at 28° C. with a relative humidity of about 50 per cent.

These experiments proved that infected lice could be recovered from patients in the epidemic area.

The possibility that bed bugs were also concerned in transmission was

TABLE I.  
TRANSMISSION OF RELAPSING FEVER FROM INFECTED LICE TO ANIMALS.

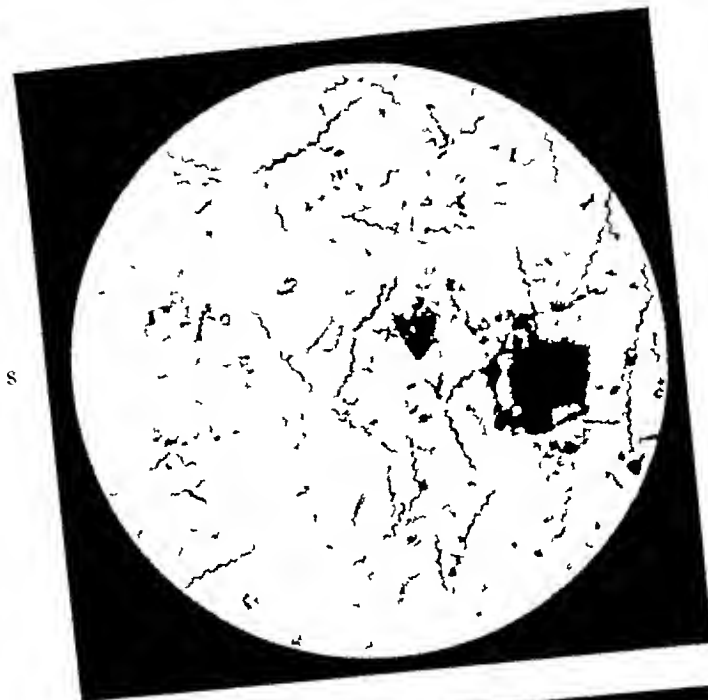
Serial number	Animal.	Lice.		Type of suspension.	Result.
		Number	Days since removal from patient.		
M9	Monkey	10	8 days	Normal saline	Transmission on 6th day
M12	—	27	8		Transmission on 4th day
M13	White mouse		8		Negative. Mouse proved susceptible by blood inoculation 3 weeks later
M8	Monkey	25	13		Transmission on 9th day
M121			13		Negative. Monkey proved unsuceptible by blood inoculation 3 weeks later (as possibly innocent)

considered. Bugs were collected from huts where cases of the disease had occurred they were dissected and smears of the organs and haemocoelic fluid were examined by dark ground illumination. No spirochaetes were seen and animal inoculations were also negative. The floors of fifty infected huts were searched for *Ornithodoros* ticks none were found.

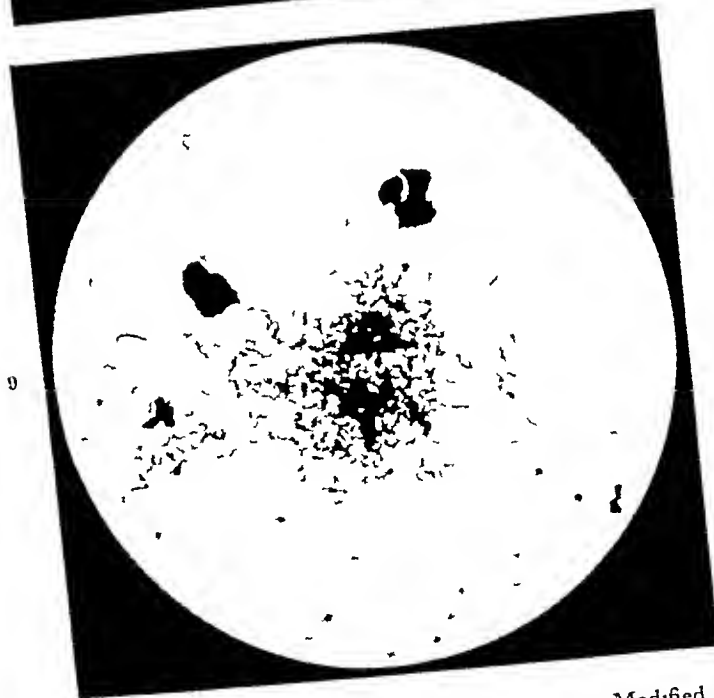
These observations appeared to prove that the epidemic was of louse-borne origin.

#### THE TYPE OF SPIROCHAETE.

The differentiation of species of relapsing fever spirochaetes is still *sub judice*.



8



9

FIG 8 Spirochaetes in a smear made from a crushed louse Modified Gordon stain  
 × 840

FIG 9 Agglutination of *S. recurrentis* Thick drop Giemsa stain × 840



## BEHAVIOUR IN EXPERIMENTAL ANIMALS.

The susceptibility of different animals to the various strains of spirochaetes is regarded by many workers as a useful criterion for diagnosis, and Table II gives a summary of results of louse-borne strains compiled from various sources. Unfortunately no standardized techniques have been employed, and without knowledge of the exact amount of the spirochaete inoculum and of the species of animal, it is impossible to assess the results satisfactorily.

However a characteristic picture was given by our animal experiments which served at least to differentiate *S. duttoni* and *S. recurrentis*. Monkeys, white rats, white mice, rabbits, guinea-pigs, bush babies and gerbilles were employed and it was found that all the animals proved susceptible to infection.

TABLE II.

BEHAVIOUR OF LOUSE-BORNE SPIROCHAETAE IN EXPERIMENTAL ANIMALS.  
(COMPILED FROM ABOVE SOURCES.)

Species of spirochaete	Monkey	Monkey to monkey	Rat.	White mouse	Mouse to mouse	Rabbit.	Guinea-pig
<i>S. recurrentis</i> (European strain)	Susceptible	Positive	Usually susceptible	Usually susceptible	Positive	Negative	Negative
<i>S. berberis</i>		Negative	With difficulty	With difficulty	Negative or with difficulty	Susceptible	Slightly susceptible
<i>S. carteri</i>		Positive	Susceptible	Susceptible	Positive	?	?
<i>S. macei</i> — Abyssinian strain			Negative			?	?
Chinese		?	?		Negative Positive	Negative	Negative

Monkeys and mice were the most susceptible, though the infection in mice was short. NICOLLE and ANDERSON (1927) stressed the effect of the size of the infective dose on both the length of the inoculation period and the character of the disease: our results were similar. Monkey 28 (Table VI page 182) showed an incubation period delayed till the 7th day following inoculation with presumably very sparsely infected cerebrospinal fluid. The three louse-transmitted infections (Monkeys 6, 9 and 12 in Table VI) also showed a long incubation, probably because the spirochaetes in the lice emulsions were scantier than those in the blood inoculations. Experiments with monkeys

These names (*duttoni* and *recurrentis*) are used to designate the tick-borne and louse-borne species respectively. It might be more accurate to refer to our East African tick-borne spirochaete as *S. recsi*.

using graduated doses were unsuccessful in confirming these results presumably because the lowest dose (0.1 c.c. of highly infective blood) was well above the minimal dose needed to show an effect.

The results of the animal inoculations are given in Tables III, IV and VI. The reaction in animals in ascending order of susceptibility is shown below —

**Rabbit**—Of five inoculated with *S. recurrentis* one became positive and exhibited spirochaetes for 2 days. Two *duttoni* inoculations failed to show organisms, but the blood of the animals when sub-inoculated into mice on the 7th day gave rise to persistent infections in the mice. No such phenomenon occurred in the *recurrentis*-inoculated rabbits.

**Guinea-pig**—One guinea-pig (No. 30) out of five showed an evanescent infection of *S. recurrentis*, with *S. duttoni* they were slightly more susceptible, one out of three becoming infected, for two days instead of one, even with a small infecting dose. KIRK (1938) and WOLMAN and WOLMAN (1945) failed to infect these animals with the Abyssinian strain.

**Bush-baby** (*Galago crassicaudatus lasotis* Peters)—Two of these animals (wild) were inoculated with patients' blood containing *S. recurrentis*, and both exhibited spirochaetes on the following day.

**White rat**—These animals behaved similarly to bush-babies, showing spirochaetes for 1 day only. KIRK (1938) failed to infect them with the Abyssinian strain. With *S. duttoni* the picture was strikingly different in that the primary infection often lasted nearly a fortnight.

**White mice**—Infections of mice with *S. recurrentis* were remarkably uniform and constant, the spirochaetes appearing on the day following inoculation and persisting for 2 or 3 days (Table IV). Relapses never occurred. In view of KIRK's complete failure to effect passage from mouse to mouse, the experiment was repeated here with five mice and sub-inoculations were performed on alternative days. Repeated passage for 80 days demonstrated how easily the strain could be maintained in the laboratory and though occasionally one or two mice in the five had occult infections, the spirochaetes invariably returned in a visible form in the next passage. This is in direct contrast to the results obtained with the Abyssinian and North African strains. Passage was effected by cutting off the head of the mouse over a Petri dish, collecting the blood (about a cubic centimetre) in a syringe and inoculating this intraperitoneally. Sterile precautions were unnecessary. The Swiss strain of mouse was used in the thirty-fourth to thirty-sixth passages, the remaining passages were in a mixed strain of mouse. *S. duttoni* infections in mice ran a very different course, being much more persistent and irregular. There were gaps of several days with absence of organisms, presumably representing intervals between relapses, and the infection sometimes lasted for 26 days. These results indicate the unique suitability of rodents as reservoirs of *S. duttoni*. HINDLE (1935) pointed out that small rodents are undoubtedly the most important carriers of the disease.

The tendency to neurotropism has been used for differentiating species of spirochaetes, and KRITSCHESKI and BRUSSIN (1931) found that *S. duttoni* was highly neutropic whereas a louse-borne Russian strain was not. KIRK (1938) likewise found no residual brain infection in mice in the Abyssinian louse-borne disease. Table V gives our results, comparing the residual effect of *S. recurrentis* and *S. duttoni* in mice. In the case of the former, there was little evidence of neurotropism, whereas with *duttoni*, although brain smears were negative, emulsions when inoculated into five animals infected three of them were negative.

**Gerbille** (*Tatera brantsi*)—Two of these animals were infected with *S. recurrentis* and spirochaetes were found from the 2nd to the 4th days of the disease. No relapses occurred.

**Monkeys**—The following species of monkeys were employed —

- Cercopithecus mitis albotorquatus* Pousargues
- Cercopithecus mitis kochi* Neumann
- Cercopithecus aethiops johnstoni* Pocock
- Cercopithecus aethiops centralis* Neumann
- Papio doguera furax* Elliot

## BEHAVIOUR IN EXPERIMENTAL ANIMALS.

The susceptibility of different animals to the various strains of spirochaetes is regarded by many workers as a useful criterion for diagnosis, and Table II gives a summary of results of louse-borne strains compiled from various sources. Unfortunately no standardized techniques have been employed, and without knowledge of the exact amount of the spirochaete inoculum and of the species of animal, it is impossible to assess the results satisfactorily.

However a characteristic picture was given by our animal experiments which served at least to differentiate *S. duttoni* and *S. recurrentis*. Monkeys, white rats, white mice, rabbits, guinea-pigs, bush babies and gerbils were employed and it was found that all the animals proved susceptible to infection.

TABLE II.  
BEHAVIOUR OF LOUSE-BORNE SPIROCHAETES IN EXPERIMENTAL ANIMALS.  
(COMPILED FROM ABOVE SOURCES.)

Species of spirochaete	Monkey	Monkey to monkey	Rat.	White mouse.	Mouse to mouse.	Rabbit.	Guinea-pig
<i>S. recurrentis</i> (European strain)	Susceptible	Positive	Usually susceptible	Usually susceptible	Positive	Negative	Negative
<i>S. duttoni</i>		Negative	With difficulty	With difficulty	Negative or with difficulty	Susceptible	Slightly susceptible
<i>S. cuniculi</i>		Positive	Susceptible	Susceptible	Positive	?	?
<i>S. morsy</i>			Negative		Negative	?	?
Abyssinian strain					Positive	Negative	Negative
Chinese		?	?				

Monkeys and mice were the most susceptible, though the infection in mice was short. NICOLLE and ANDERSON (1927) stressed the effect of the size of the infective dose on both the length of the incubation period and the character of the disease: our results were similar. Monkey 28 (Table VI page 162) showed an incubation period delayed till the 7th day following inoculation with presumably very sparsely infected cerebrospinal fluid. The three louse-transmitted infections (Monkeys 6, 9 and 12 in Table VI) also showed a long incubation, probably because the spirochaetes in the lice emulsions were scantier than those in the blood inoculations. Experiments with monkeys

These names (*duttoni* and *recurrentis*) are used to designate the tick-borne and louse-borne species respectively. It might be more accurate to refer to our East African tick-borne spirochaete as *S. rusei*.

using graduated doses were unsuccessful in confirming these results presumably because the lowest dose (0.1 c.c. of highly infective blood) was well above the minimal dose needed to show an effect.

The results of the animal inoculations are given in Tables III, IV and VI. The reaction in animals in ascending order of susceptibility is shown below—

**Rabbit**—Of five inoculated with *S. recurrentis* one became positive and exhibited spirochaetes for 2 days. Two *duttoni* inoculations failed to show organisms, but the blood of the animals when sub-inoculated into mice on the 7th day gave rise to persistent infections in the mice. No such phenomenon occurred in the *recurrentis*-inoculated rabbits.

**Guinea pig**—One guinea pig (No. 30) out of five showed an evanescent infection of *S. recurrentis*, with *S. duttoni* they were slightly more susceptible, one out of three becoming infected, for two days instead of one, even with a small infecting dose. KIRK (1938) and WOLMAN and WOLMAN (1945) failed to infect these animals with the Abyssinian strain.

**Bush-baby** (*Galago crassicaudatus lasotis* Peters)—Two of these animals (wild) were inoculated with patients' blood containing *S. recurrentis*, and both exhibited spirochaetes on the following day.

**White rat**—These animals behaved similarly to bush-babies, showing spirochaetes for 1 day only. KIRK (1938) failed to infect them with the Abyssinian strain. With *S. duttoni* the picture was strikingly different in that the primary infection often lasted nearly a fortnight.

**White mice**—Infections of mice with *S. recurrentis* were remarkably uniform and constant, the spirochaetes appearing on the day following inoculation and persisting for 2 or 3 days (Table IV). Relapses never occurred. In view of KIRK's complete failure to effect passage from mouse to mouse, the experiment was repeated here with five mice and sub-inoculations were performed on alternative days. Repeated passage for 80 days demonstrated how easily the strain could be maintained in the laboratory and though occasionally one or two mice in the five had occult infections, the spirochaetes invariably returned in a visible form in the next passage. This is in direct contrast to the results obtained with the Abyssinian and North African strains. Passage was effected by cutting off the head of the mouse over a Petri dish, collecting the blood (about a cubic centimetre) in a syringe and inoculating this intraperitoneally. Sterile precautions were unnecessary. The Swiss strain of mouse was used in the thirty-fourth to thirty-sixth passages, the remaining passages were in a mixed strain of mouse. *S. duttoni* infections in mice ran a very different course, being much more persistent and irregular. There were gaps of several days with absence of organisms, presumably representing intervals between relapses, and the infection sometimes lasted for 26 days. These results indicate the unique suitability of rodents as reservoirs of *S. duttoni*, HINDLE (1935) pointed out that small rodents are undoubtedly the most important carriers of the disease.

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**Gerbill** (*Tatera brantsi*)—Two of these animals were infected with *S. recurrentis* and spirochaetes were found from the 2nd to the 4th days of the disease. No relapses occurred.

**Monkeys**—The following species of monkeys were employed—

*Cercopithecus mitis albotorquatus* Pousargues

*Cercopithecus mitis kochi* Neumann

*Cercopithecus aethiops johnstoni* Pocock

*Cercopithecus aethiops centralis* Neumann

*Papio doguera furax* Elliot

TABLE III  
RESULTS OF INTRAPERITONEAL INOCULATION OF BLOOD CONTAINING *S. marseus* ( ) AND *S. dysenteriae* ( ) INTO ANIMALS VARIALLY

Retal number	Animal	Origin and percent	Daily blood examination.															Remarks.
			3	4	5	6	7	8	9	10	11	12	13	14	15			
1	Guinea pig	Human blood	R													2 c.c. blood inoculated		
2			R													3 c.		
120			R														10 c.c.	
54			R														15 c.	
48			R														8 c.c.	
40	Rabbit	Monkey blood	D													Second attempt		
5			D													1 c.c. blood inoculated		
170			D														No infection	
171			R															
101			R															
112	Human blood	Human blood	R															
86			R															
87			D															
			D															
			D															
16	White	Human blood	R															
14			R															
18			R															
88			D															
89			D															
119	Black-baby	Human blood	D															
120			D															
11			R															
111			R															
112			R															

R = *S. marseus*D = *S. dysenteriae*

2 c.c. blood inoculated  
3 c.  
10 c.c.  
15 c.  
8 c.c.  
Second attempt  
1 c.c. blood inoculated  
No infection  
Occult infection in rabbits  
Accidental death  
Demonstration of virulence after mouse passage  
Died from septicaemia

3 c.c. blood inoculated  
3 c.  
10 c.c.  
15 c.  
8 c.c.  
Second attempt  
1 c.c. blood inoculated  
No infection  
Occult infection in rabbits  
Accidental death  
Demonstration of virulence after mouse passage  
Died from septicaemia

3 c.c. blood inoculated  
3 c.  
10 c.c.  
15 c.  
8 c.c.  
Second attempt  
1 c.c. blood inoculated  
No infection  
Occult infection in rabbits  
Accidental death  
Demonstration of virulence after mouse passage  
Died from septicaemia

3 c.c. blood inoculated  
3 c.  
10 c.c.  
15 c.  
8 c.c.  
Second attempt  
1 c.c. blood inoculated  
No infection  
Occult infection in rabbits  
Accidental death  
Demonstration of virulence after mouse passage  
Died from septicaemia



Most of the monkeys were wild but two were laboratory-bred (one was used for successful house transmission experiment and the other for blood inoculation). No natural infections with spirochaetes were found in the wild monkeys, nor in an additional twenty-five used for other work. WOLMAN and WOLMAN (1945) state that the response of monkeys to infection is so variable that results are quite unreliable. This was not our experience, for as NICOLLE and ANDERSON (1937) found in Tunis, even different species of monkeys reacted in a very constant manner. Monkeys were the only animals which became febrile on infection. (The temperatures of all animals were taken daily). A raised temperature did not occur in every case and appeared to depend upon the intensity of the infection. The temperature became sub-normal for day or two before death in the *duttoni* cases. The incubation period in *S. recurrentis* was usually 1 to 3 days after blood inoculation after infection from lice 6 to 7 days. *S. duttoni* in monkeys

TABLE V  
NEUTROPHILS IN *S. recurrentis* AND *S. duttoni* INFECTIONS IN WHITE MONKEYS

Serial number	Length of infection in blood	Spirochaeta	Relapse	Date sacrificed	Direct examination of brain smears	Result of inoculation of brain smears into animals
20	3 days	R	N2	21st day	Negative	Negative (Mouse No. 31)
24	2	R		Died 2nd day	Scanty spirochaetes	Not done
27	2	R		7th day	Negative	Negative (Mouse No. 32)
63	2	R		30th		( No. 192)
64	2	R		30th		( No. 193)
65	2	R		30th		( No. 194)
66	2	R		30th		( No. 195)
48	8 days	D	N2	25th day	Negative	Negative (Monkey No. 40)
76	17	D	Ta	23th		Not done
77	17	D		23th		Positive (Mouse No. 207)
78	17	D		23th		( No. 208)
79	13	D		61st		( No. 224)
80	15	D		61st		Negative (Mouse No. 225)

R = *S. recurrentis*.

D = *S. duttoni*.

had rather longer incubation period (about 8 days). Relapses occurred in thirteen out of nineteen *recurrentis* infections with fairly constant 10- to 14-day periodicity. The first relapse of *recurrentis* tended to be longer and more febrile than in the original attack (rifle Nos. 9, 12 and 23, Table VI), and in three cases the monkey died during the relapse (rifle Nos. 34, 43 and 144). Only one case had more than one relapse. KNOT's experience with the Abyssinian strain was that there was never more than a single relapse; that the incubation period in house infections was 4 days and that some of the latter proved fatal. *S. duttoni* infections behaved very differently: of ten infected monkeys, all died (see

There was one exception. *C. mitis hanksi* survived *S. duttoni* infections whereas all other species died.

Tables VI and IX) Monkey 50 was treated with acetylarsan, which cured the primary attack, though the animal succumbed during the first relapse. The periodicity here (and in a later case) was 14 days. Passage from monkey to monkey was successful on numerous occasions.

The results of these animal experiments indicate that our louse-borne strain corresponded fairly closely with *S. carteri* rather than with *recurrentis* or *berbera*. It was definitely unlike the Abyssinian strain. Comparison with *S. duttoni* showed the latter's completely different reaction in mice, rats and monkeys (Table VII).

#### VARIATION IN VIRULENCE OF SPIROCHAETES AFTER PASSAGE

Little variation in virulence was observed in the strains of the spirochaetes obtained in the epidemic. There was one exception. This occurred in the case of an Arab taken off a dhow arriving at Mombasa from the Hadhramaut, several monkeys (e.g., M 43) inoculated or passed from his blood died during the first relapse, whereas no fatalities had occurred before. Repeated passage through mice did not apparently affect the virulence (e.g., M 144). With *S. duttoni*, however, although passage through mice was unable to prevent a fatal termination in monkeys (*vide* M 143), it did appear to shorten the infection in rats (199 and 120).

#### BEHAVIOUR IN ARTHROPODS

Division into tick-borne and louse-borne strains appears now to be accepted as the most reasonable way of classifying the relapsing fevers. The experiments described above (page 153) showed that the Kenya epidemic was louse-borne. A further interesting point arises. Recent observations on louse-borne strains by WOLMAN and WOLMAN (1945) in Abyssinia, and CHUNG and FENG (1936) in China, suggested that at no time during the cycle in the louse do spirochaetes completely disappear and that they can actually be found every day in a proportion of the insects. KIRK (1938), while working with the Abyssinian strain in the Sudan, found spirochaetes in 15 to 20 per cent of recently fed lice (examined after 24 hours), thus confirming to some extent the above observations. Now these results conflict with the classical picture as described by NICOLLE, BLAIZOT and CONSILIL (1912) and CHAPCHEFF (1925), who found a long negative phase (8 and 11 days respectively) in the louse, and it is interesting to note that the Kenya strain also showed this negative phase. BURTON (1939) states that spirochaetes are found in the louse (at 28° C.) from about the 6th day; MANSON-BAHR (1943) quotes the 16th day. The cycle here was worked out in the following way:—

Lice were collected from infested blankets in a clean area (Nairobi) and were sent by train to Mombasa where they were fed on patients suffering from relapsing fever. They were then returned to Nairobi and kept alive by feeds twice daily on ourselves. The first batches had a high mortality and all died





Tables VI and IX)  
attack, though the a  
in a later case) was  
occasions

occasions		Daily blood examination														Remarks
The re	strain cor	18	19	20	21	22	23	24	25	26	27	28	29	30		
or ber <sup>t</sup>																
S	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
—	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
—	+	+	+	+	—	—	—	+	+	+	—	—	—	—		
—	+	+	+	+	—	—	—	—	—	—	—	—	—	—		
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
nd pathology of organs	—	+	+	+	—	—	—	—	—	—	—	—	—	—		
—	+	+	+	—	—	—	—	—	—	—	—	—	—	—		
+	+	+	—	—	—	—	—	—	—	—	—	—	—	—		
—	+	+	+	+	—	Died of disease in first relapse								Relapse 10th day 12th		
+	+	+	—	—	—	—	—	—	—	—	—	—	—	—		
re	+	+	+	+	Died in first relapse								Relapse 11th day			
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
+	—	+	—	—	—	—	—	—	—	—	—	—	—	—		
+	+	+	Died after cardiac puncture											"		
icture	—	—	+	+	+	+	+	+	+	+	+	Died in first relapse		12th		
7th day	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
usease on 8th day	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
on 5th day	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
—	—	—	—	—	+	+	+	Died of disease in first relapse						Acetylarsan 0.016 g on 9th day		
6th day	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

R = *S. recurrentis* D = *S. duttoni*

Unlike KIRK (1938) and ROBERTSON (Chinese strain, 1932) we were never able to find spirochaetes in lice taken from patients and examined within 24 hours

It is suggested, therefore, that the louse-borne spirochaetes of man can be divided into two groups —

- 1 Spirochaetes with a long negative phase in the louse (Lice collected from patients usually negative when examined within 24 hours) Europe, North Africa, Kenya

2. *Spirochaetes* present throughout the louse cycle. (Lice collected from patients often show *spirochaetes* when examined within 24 hours) China Abyssinia.

In order to confirm that the Kenya infection belonged to the *recurrentis* and not to the *duttoni* group its transmissibility by ticks (*Ornithodoros moubata*, four batches, and *savignyi* one batch) was tested. Ticks were obtained from a year-old laboratory colony the nymphs were fed on heavily infected patients or monkeys and were kept at 28° C. until a week before examination, when they were transferred to the 37° C. incubator. Coxal fluid and emulsified contents all proved negative on examination and the latter failed to infect monkeys. It will be recalled that Kink (1933A) failed to transmit the Abyssinian strain with *O. savignyi*.

TABLE VII

DIFFERENTIATION OF *S. recurrentis* AND *S. duttoni* BY EFFECT OF INOCULATION INTO MAMMALS

Animal	<i>S. recurrentis</i>	<i>S. duttoni</i>
Monkey	Moderate infections. 60 per cent. show relapses. Rarely fatal in primary attack.	Very severe and almost uniformly fatal infection in primary attack.
White mouse	Infection on 2nd and 3rd days. No relapses.	Variable incubation period. Many relapses at short intervals.
White rat	Infection lasts one day only	Infection very persistent, usually lasting for week or longer

### IMMUNITY REACTIONS

Unfortunately we had in our possession only two strains of *spirochaetes*—*duttoni* and *recurrentis*. Cross immunity experiments with monkeys clearly differentiated these strains as shown in Table IX. Monkeys which had recovered from *recurrentis* infections all died when inoculated with *duttoni* blood, showing a complete lack of immunity to the latter species, whilst when inoculated with *recurrentis* blood either no infection or a very mild one resulted. Immunity against the homologous strain of *recurrentis* persisted for at least 2 months.

The relatively refractory bush-baby proved immune to *S. duttoni* after recovery from *S. recurrentis* infection. White rats, however like monkeys, showed an absence of cross immunity and after recovery from *recurrentis* responded to *duttoni*.

The almost consistently fatal nature of *duttoni* in monkeys made it impossible to test cross immunity of this species against *recurrentis*. Mice, however which had recovered from *duttoni* were found to respond normally to *recurrentis*.

CHEN, ZIA and ANDERSON (1945) found, on the contrary that cross

TABLE VIII  
DEVELOPMENT OF SPIROCHAETE INFECTION IN LICE.

Days after feeding on infected patient	Number examined	Result
2nd		
3rd	20	All negative
6th	3	"
7th	3	"
8th	6	"
9th	6	"
10th	6	"
11th	0	"
12th	6	Five negative, one very scanty spirochaetes
13th	6	All negative
14th	6	"
15th	6	"
16th	6	"
17th	6	"
18th	6	Four negative, two numerous spirochaetes
19th	6	One " " " "
	6	" " " " " "
20th	6	Four " " " " " "
	5	spirochaetes " " " " " "
		Four negative, one numerous spirochaetes

Twenty of the progeny of the infected lice were kept alive for 3 weeks and were found negative on examination

immunity occurred in monkeys between infections with a Californian tick-borne strain and the louse-borne Chinese strain

Table IX also shows the effect of re-inoculation with a first relapse strain of *recurrentis* and the existence of cross immunity in two out of three monkeys. Monkey 5, however, which a fortnight before had been shown to be immune to a primary strain, was susceptible to a first relapse strain

No serological reactions were tested, though the phenomenon of auto-agglutination of spirochaetes in the blood of old infections was frequently observed. For instance, Monkey 97 (Table VI) exhibited spirochaetes for the exceptionally long period of 6 days, on the 5th and 6th days, masses of tangled organisms (Fig 9, facing page 154) were seen in thick drop preparations of blood. On the other hand, white rats infected with *duttoni* even on the 10th or 11th day of the disease failed to show this phenomenon, presumably because this species of animal is incapable of producing agglutinins. Agglutination of spirochaetes in monkeys is only seen after the blood has been outside the body for several

minutes and is therefore observable only in thick drop or wet preparations and not in thin smears, which dry rapidly.

One monkey (M4) was splenectomized a fortnight after its primary infection with *S. recurrentis*. No relapse followed, and a month later it was immune to a relapse strain.

Infected blood which had been citrated and in which the spirochaetes were presumably dead, appeared in some instances to immunise the animals against the homologous strain. A white mouse and a white rat were inoculated with citrated blood from a white rat containing many spirochaetes. No infection

TABLE  
CROSS IMMUNITY IN MONKEYS

Serial number	History of first infection.		Date of re-inoculation.	Species of re-inoculation.
	Spirochaete.	Length, including relapse.		
3	<i>recurrentis</i>	4 day	31 day later	<i>Autum</i>
4		4	66	
9		29	33	
14		10	40	
12		13	73	
12		13	63	<i>Hyattii</i>
7		4	63	
8		3	62	
6		13	84	
9		3	67	
6		3	83	First relapse strain
4		4	27	
3		13	71	

resulted and the animals proved immune to re-inoculation of fresh infective blood a fortnight later. The experiment was repeated, and it was found that two out of five mice showed similar results. Another mouse was successfully immunized by a *recurrentis* suspension, prepared by STURV's (1944) method.

#### MORPHOLOGY

It is well recognized that all relapsing fever spirochaetes resemble each other closely and no differentiating characters appear to exist. Examination by dark ground illumination revealed the well-known corkscrew appearance but in some human cases practically all the organisms seemed to consist of a twisted chain of tiny beads with clear spaces intervening (the coccoid bodies as described by THOMSON and ROBERTSON 1929). Spirochaetes in smears

made from frozen citrated blood (KIRK, 1938) were measured and the average length was found to be  $16\mu$

Some of these spirochaetes from the frozen blood remained motile for at least 48 hours and were capable of infecting mice

Dilution of infective blood with water caused no loss of motility of the spirochaetes until sufficient water had been added to produce haemolysis, at that point the organisms became immobile and were apparently dead Some of our earlier animal experiments were ruined by using water instead of normal saline for dilution purposes

## IX.

*S. recurrentis* AND *S. duttoni*

Daily blood examination after reinoculation														Remarks
2	3	4	5	6	7	8	9	10	11	12	13	14		
—	—	—	+	+	+	+	+	Die d						No cross immunity
—	—	+	+	—	—	+	+	+	Die d					
+	+	+	+	+	+	Die d								"
—	—	+	—	—	—	Die d								
—	+	+	—	—	—	—	—	Die d						"
—	—	+	—	—	—	—	—	—						
—	—	—	+	+	+	—	—	—						M 12 was immune to recurrentis
—	—	—	—	—	—	—	—	—						
—	—	—	—	—	—	—	—	—						Mild infection with homologous strain
+	+	+	—	—	—	—	—	—						
														Immunity lost to homologous strain
														Immune to homologous strain
														"
														first relapse strain
														Susceptible to first relapse strain

Division by transverse fission was observed once in certain blood films, and particularly in the effect of longitudinal division was observed in the

Division by transverse fission was observed once in a wet preparation In certain blood films, and particularly in lice smears, appearances very suggestive of longitudinal division were encountered, but these were undoubtedly the effect of twisting

In lice the spirochaetes usually appeared much more delicate and maintained their corkscrew form Serial sections stained by the Levaditi method revealed their localization in the thoracic muscles and the abdomen They were absent from inside the gut and from the mycetome

The following conclusions may now be drawn in regard to the identity of the spirochaete responsible for the Kenya epidemic

Origin Southern Arabia Vector The louse  
The clinical picture in man and animals resembles that of *S. carteri* The

easy passage in mice and the absence of a negative phase in the louse differentiates the Kenya spirochaete from the Abyssinian and Chinese strains, whilst its behaviour in mice also appears to distinguish it from *S. berberis*.

Cross immunity animal and transmission experiments demonstrated that the spirochaete responsible for the epidemic was not *S. duttoni*.

The only valid criterion for separating the louse-borne strains (*S. recurrentis*) appears to be the presence or absence of a negative phase in the louse (see page 163) the Kenya spirochaete has a negative phase.

### SUMMARY

1 An epidemic of relapsing fever involving the hinterland of Mombasa and the Kenya Coast is described.

2 There were nearly 2,000 cases, with a 40 per cent. mortality in untreated cases.

3 Control measures rapidly terminated the epidemic. About 100,000 people were disinfested with 5 per cent. DDT powder. Various administrative steps (such as abolition of funeral ceremonies, prevention of travel, etc.) were also important in the control of the spread of the disease.

4 Clinically cases were characterized by the prominence of neurological symptoms, cardiac involvement, positive Weil Felix reactions and negative Kahns.

5. The disease was introduced from Arabia and was of louse-borne origin. Infected lice were recovered from patients and the disease was transmitted from these lice to monkeys. There was no evidence incriminating other possible vectors and the spirochaete was found to be non transmissible by the ticks *O. moubata* and *sergenti*.

6. The behaviour of the spirochaete in different animals was observed and compared with that of *S. duttoni*. Rabbits, guinea-pigs, bush-baboes, white rats, white mice, gerbilles and monkeys were found capable of being infected, their relative susceptibility being in the order shown, with rabbits the least and mice and monkeys the most susceptible. The following observations are particularly to be noted —

(i) Repeated passage in mice was easily maintained.

(ii) Neurotropism occurred in *S. duttoni* infections in mice but scarcely ever in *recurrentis*.

(iii) Relapses of *S. recurrentis* occurred in 69 per cent. of monkeys, the interval between the relapses being 10 to 12 days. Relapses tended to be more severe than the original attack.

(iv) *S. duttoni* infections differed from *recurrentis* in being much more fatal in monkeys and much more persistent in rats and white mice.

7 The infections in lice showed a well marked negative phase lasting till the 16th day. Lice collected from patients and examined within 24 hours never showed spirochaetes.

8 No cross immunity exists in animals (except bush-babies) between *S recurrentis* and *S duttoni*. There was cross immunity in two out of three monkeys tested against a relapse strain.

9 Auto-agglutination of spirochaetes in old infections (primary attacks) occurs in monkeys but not in rats.

10 Some evidence was produced to show the immunizing effect of killed spirochaetes.

11 The pathogenicity of the Kenya spirochaete in man and animals resembles most closely that of *S carteri* and differs markedly from the Chinese and Abyssinian strains.

12 It is suggested that the louse-borne spirochaetes may be divided into two groups. The first exhibits a long negative phase in the louse (European, North African and Kenya strains), the second never shows this phase and spirochaetes are present throughout the louse cycle (Abyssinian and Chinese strains).

13 The pathological changes resembled those previously described except that in three instances degeneration of ganglion cells was found in the cerebellum without meningovascular inflammation.

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# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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In an article by the Senior Medical Officer Grenada, on the Diet of the Mental Worker in the Tropics (Caribbean Medical Journal 1943 128) reference was made to the conditions associated with 11 in tropical regions. The effect of adding Vitamin B complex to normal diet was tested on ten subjects whose work involved certain amount of concentration and mental effort. With one exception all subjects benefited in their ability to concentrate, bile dyspeptic disorders, heartburn, constipation, were either banished or were lessened. Appetite improved, and sleep was less broken and more refreshing.

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**TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE**

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**VOL 41 No 2 OCTOBER, 1947**

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**THE  
FORTIETH ANNUAL GENERAL MEETING**

of the Society held at

**Manson House, 26, Portland Place, London, W 1,**

on

**Thursday, 19th June, 1947, at 8 p m**

**Dr C M WENYON, C M G , C B E , F R S (PRESIDENT)**

in the Chair, followed by

**SIR PHILIP MANSON-BAHR, C M G , D S O , F R C P (the new PRESIDENT)**

---

**B U S I N E S S .**

---

**REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1947**

The **Hon Secretary**, Prof N HAMILTON FAIRLEY, presented the Annual Report, which had been circulated before the meeting to those present. He spoke of the very satisfactory nature of the Report generally.

The President asked if anyone wished to make any comment on the Annual Report.

Dr L E Napier. I think this Annual Report is extremely satisfactory as far as it goes but it is a little disappointing as a Report of the Council. I feel that in these democratic days the members of the Society should be taken more into the confidence of the Council. There are many Council meetings throughout the year, they are well attended and sometimes go on so long as

to make us a little late for our dinner and I cannot believe the entire time is spent on details of whether the meeting shall be on the first, second or third Thursday of the month. Beyond that there are of course the names of proposed new Fellows which will no doubt take some but not a great deal of time to consider. This should leave a considerable margin of time for discussion on the policy of the Society. There was a meeting about 18 months ago, at which I was honoured by being asked to open a discussion on the teaching of tropical medicine. It was a very interesting discussion and many people took part in it. I made two specific proposals and, although many people differed with a great deal that I had said, nobody disagreed with those proposals. At the end of the meeting I heard that the Council would discuss the proposals, but from that day to this the only allusion to them that I know of consists of two lines in last year's Annual Report. The Council in February appointed a Tropical Medicine Policy Committee with Dr GEORGE MACDONALD as its Secretary. That is the only reference to that discussion on that particular subject which has been given to the members. I personally have not received any other information beyond this. We do not know who the members of the Committee were. We do not know if they ever met, or what they discussed or decided. If they never met the members should at least be told that the whole thing has fallen through. But perhaps there were many meetings.

My second point has reference to the Aims of the Society as expressed on page 1 of the *Year Book*. (This red book is incidentally the most valuable book published in connection with tropical medicine because it contains everybody's address.) The first aim of the Society is set out in a short paragraph to stimulate "enquiry and research into the causes, treatment and prevention of human and animal diseases in warm climates by facilitating the publication and discussion. That is fulfilled admirably by this Society better than by any other Society in the world. The American Society of Tropical Medicine is in a less fortunate position in not having a headquarters like we have. They cannot have frequent meetings like we have here. The papers read here are most valuable and the discussions that follow are often more valuable. But I feel that the Society could do very much more with reference to the second point suggested in these aims, that is by promoting social intercourse among scientific workers in all parts of the tropical world. Members of Council cannot rush off in all directions and encourage social intercourse among scientific workers throughout the world. But they could do more than is done now in encouraging social intercourse amongst tropical workers when they are in this country. Those of us who live in England enjoy these meetings very much indeed. We meet here once a month, and since I have been in the country I have not missed a single meeting unless perhaps the first one because I was not back in time to receive the notice. But what do we do for Fellows still engaged in the tropics? One doctor here tonight came home 10 days ago, just in time for this Annual General Meeting. There will now be a blank of

## ANNUAL GENERAL MEETING

3 months with no meetings at all. If his leave is prolonged he may possibly get to one meeting before he goes back in October. We shut down completely just at the time when the tropical worker is amongst us. I feel that is wrong, and that even if we gave up meetings during other parts of the year, we should have a full number of meetings during the summer. The climate of London does not preclude this. We had the only heat wave we shall probably get this year about a month ago. I think that not only should there be meetings but this place should be a centre where people working in the tropics could come and meet at any time of the day. I would not suggest anything so drastic as converting it into a club with a bar, but it should be much more of a social centre. We come back on leave, some of us have a club in London where we can meet a few old friends, but we do not meet other tropical workers. We should not only have evening meetings, but possibly afternoon meetings, with less formal discussions, although admittedly our present discussions are not unduly formal. There should also be somebody prepared to spend time finding out who is in England, and when they come to this building everything should be done to entertain them and see that they meet one another. As it is, one comes here without any idea of who is in England. If there were a list put up in the Society's rooms and Fellows were asked to give notice of when they would be in England and London, it would provide an opportunity for people to meet one another and it would promote social intercourse among scientific workers from all parts of the tropical world. If the Society did not feel that its scope went as far as that, I would suggest the formation of a club within the Society. We might have a Manson Club for the purpose of arranging dinners and even less formal meetings than the ordinary monthly meetings of the Society.

I don't know if I have spoken at the right time, but I felt that I had to say this and I have said it.

**The President, Dr C M Wenyon** We are very much indebted to Dr Napier for his comments on the policy of the Society. I think his remarks about the Annual Report rather suggest that it is his idea that more detail of the proceedings of Council should be in the Annual Report for the information of the Fellows generally. There may be something in this, and possibly on a future occasion somebody may have the spare time to devote to these extensions of the Annual Report. As regards policy, I wonder if Dr NAPIER understands clearly what the workings of the Society have been. He has spent many years in India, but was not during that time a Fellow of the Society so that as far as the Society is concerned Dr NAPIER is a newcomer. He has spent some time in America since leaving Calcutta and has seen something of the American Society of Tropical Medicine over there, which is handicapped by not having a central headquarters as this Society has. Many of the suggestions made by Dr NAPIER have already been discussed in the Council on more than

one occasion, but the trouble is that many of us are too busy to devote more time to the Society. I sometimes feel that I have devoted more time to its service than I should have done, having been ever since 1920 Honorary Secretary and finally President.

**Dr G Carmichael Low** In answer to Dr NAPIER, I might point out that when we got this house and moved from Chandos Street the idea was—it was my idea certainly—that we should have a centre for tropical medicine, and eventually would develop part of Manson House as a club where men when they came home from abroad could stay for a time and meet other friends. We thought we might give them bed and breakfast and other facilities, but at the moment we did not have the necessary money to carry out such a plan. We have been all these many years trying to clear off our debt, which was considerable, and I am happy to say we paid it off in 1945 but to accomplish this we had to let some of the top floors of the building so as to get the necessary funds. We have discussed many times whether we would, when we got enough money develop part of the house as a club where we could have a certain number of rooms where men could, as I have said, stay for a time when they came home, and this possibility still exists for the future. It costs money to make the necessary alterations, however and that is how the matter stands at the moment. As regards men knowing when other men are home, if you look in the TRANSACTIONS you will notice that for a long time there have been lists of Fellows who are home on leave. Any man who comes home from Nigeria, say, may look up this list and see that Jones or Smith is here. He can then drop a line to Manson House and it will be forwarded to Smith a home address and so put them in touch with each other. Dr NAPIER's comments are welcome but are not entirely fair as Dr WYNTON has said. I do not think I have any need to say more. I have explained the position as it is and what the future may bring. I think there will certainly be further developments as the years pass by.

**Prof George Macdonald** Since my name has been mentioned, perhaps a word of personal explanation might be justified. During the discussion on Dr NAPIER's paper on medical education, I suggested that we might have within the Society some organization for the formulation of educational and other policy—a suggestion which was later considered by the Council and implemented by the formation of a Policy Committee. There was considerable delay in calling a meeting of this Committee, but it did meet and discussed the general framework of policy in the teaching of tropical medicine, and though it did not take any executive action it was of some use in that it served as a means of exchange and co-ordination of ideas between members of the different schools. It has subsequently expired with the expiry of the old Council, and the question of whether it should be revived is one for the new Council.

## ANNUAL GENERAL MEETING

Not as a member of the Council, but as a Fellow of the Society, I would like to join in the discussion on the extension of our facilities to include social matters. The Fellows in England get a great deal of good from the Society through their ability to attend meetings where they meet others with similar interests in a pleasant social atmosphere. If we should find ourselves financially capable of extending our activities in any way, I feel very strongly that we should do our best to increase the help we give to the Fellow overseas, and to give him some additional benefit whilst he is overseas and not merely on an occasional visit to this country. The only way in which we can do this is by an increase in our publications, either in the form of a larger TRANSACTIONS or of additional monographs on special subjects, and this should take precedence over any increase in our social or club facilities in London.

The President Professor MACDONALD suggests that we might increase the scientific publications of the Society. This suggestion has been discussed by the Council on several occasions lately, and it has been decided that when funds are available we shall devote them to extending our publications. We realize that what the Fellows abroad get from the Society are its publications, and we should do our best to make these as attractive and useful as possible. Unfortunately, our finances will not permit of anything more being done immediately. Those who study the Annual and the Treasurer's Reports and the Accounts and Balance Sheet, will see that last year the Society had a balance of income over expenditure of £38. Many of the papers we publish in the TRANSACTIONS cost the Society a great deal more than £38, so this does not leave us much latitude to increase the publications of the Society at the present time. We have great hopes that a little later the financial position may improve, and then it is our intention to do something on the lines Professor MACDONALD has suggested.

Dr. Napier proposed the adoption of the Annual Report, and Dr F Hawking seconded the resolution, which was carried.

The Hon Treasurer (Dr O MARRIOTT) read his Report. He said that it had cost £450 to publish the last number of the Society's TRANSACTIONS. As to the proposed club, perhaps he, as Hon Treasurer, had held the deciding opinion in negating that project under present conditions. The excess of income over expenditure this year was only £38. So far they had been clearing off debt and getting ready. They still had expenses, £38 was not enough to open up a large policy. Adequate funds must first be accumulated. Another point was that he had always maintained that the best value of the Society to Fellows abroad consisted in the TRANSACTIONS and whereas the TRANSACTIONS used to have only 100 pages and were cut down to some sixty or seventy pages during the war on account of paper shortage now, when



barely out of the war the last number of the TRANSACTIONS contained 218 pages of text and cost over £450. He thought this marvellous and it should be remembered that the cost of printing, blocks and paper had gone up enormously. It was to be hoped that expenditure along those lines had reached its top. The Society would have to earn some money during the next few years before launching out on anything extra. The majority of their Fellows were abroad and he felt that the Society's more useful policy was to give them the best it could for reading in Malaya, West Africa or wherever they might be.

Before sitting down he would like to again remark on the great assistance he had always received as Treasurer and previous Treasurers would bear him out in this, from the Society's former Secretary Miss WENTON who had been the right hand man of the Treasurer. By watching the affairs of the Society getting things done economically cutting down needless expenditure, she had saved the Society an enormous amount of money.

Dr W. E. COOKE proposed the adoption of the Treasurer's Report.

Dr H. M. HANSELL seconded the resolution and it was carried.

#### ELECTION OF THE AUDIT COMMITTEE

The President. The Audit Committee consists of three Fellows of the Society who are not Members of Council. They meet and consider the accounts when these are drawn up by the auditors, keep an eye on things and see that everything is above board. The present members of the Audit Committee are Dr W. E. COOKE, Dr J. C. BROOM and Dr C. A. HOARE. These gentlemen, I understand, are willing to serve again. They are eligible for election. If somebody would like to propose their election, will he do so.

Sir LEONARD ROGERS proposed this. Prof H. E. SMOLEY seconded the resolution, and it was carried.

#### ELECTION OF PRESIDENT TWO VICE PRESIDENTS AND TWENTY COUNCILLORS

The President. Dr C. M. WENTON, then announced the result of the Ballot as follows —

##### *President*

Sir PHILIP MANSON BARR, C.M.G., D.S.O. M.D. F.R.C.P. D.T.M. & H.

##### *Vice-Presidents*

P. A. BUXTON C.M.G., M.R.C.S., L.R.C.P. D.T.M. & H. F.R.S., Professor  
R. M. GORDON O.B.E. M.D., F.R.C.P., D.P.H. D.T.M., Professor

*Councillors*

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- \*JOHN BENNET, M D, M R C P, D P M, D T M & H, Brigadier (late R A M C)
- J S K BOYD, O B E, M B, CH B, D P H, D T M & H, Brigadier (late R A M C, ret)
- C C CHESTERMAN, O B E, M D, B S, M R C P, D T M & H
- T H DAVEY, O B E, M D, D T M, Professor
- N HAMILTON FAIRLEY, C B E, M D, D S C, F R C P, F R S, Professor
- \*CECIL J HACKETT, M D, B S, M R C P, D T M & H
- R BRUNEL HAWES, M B, B S, F R C P
- E H VERE HODGE, C I E, M D, F R C P, Lt-Col I M S (ret)
- W H KAUNTZE, C M G, M B E, M D, CH B, D P H, F R C P
- \*E M LOURIE, M B, B S, D P H, D T M & H
- GEORGE MACDONALD, M D, CH B, D P H, D T M, Professor
- B C MAEGRAITH, M B, B S, D P H, Professor
- \*A F MAHAFFY, C M G, M B, D T M
- \*T C MORTON, O B E, M D, M R C P, D T M & H, Air Commodore R A F
- F MURGATROYD, M D, D T M, F R C P
- H E SHORTT, C I E, M D, D S C, Col I M S (ret), Professor
- Sir JOHN TAYLOR, C I E, D S O, M D, L L D, D P H, Maj-Gen I M S (ret)
- \*F NORMAN WHITE, C I E, M D, I M S (ret)
- CHARLES WILCOCKS, M D, CH B, M R C P, D T M & H

\* New Nomination

**The President (Dr Wenyon)** We now come to the most important item of the meeting, the induction of the NEW PRESIDENT, to whom I shall have to hand over this Badge and Chain of office. It is unnecessary for me, I think, to review in detail the proceedings of the Society during my years of office. They have been reviewed to some extent, I cannot say in great detail after what has been said, in the Annual Reports for the two years of my term of service. This period has been one of clearing up after the war. Most of the structural damage to Manson House has been repaired, though a recent payment for war damage is responsible for the small balance of £38 compared with one of over £800 in last year's accounts. Fellows will be glad to learn that this payment is largely, if not entirely, recoverable from the War Damage Commission. On the subject of Manson House I am again reminded that the debt on Manson House was liquidated in May, 1945. The securing of this house for the Society is a notable achievement, and I am proud to think that Manson House, purchased in 1931 during the Presidency of Dr CARMICHAEL LOW, when I was one of the Honorary Secretaries, was brought to completion during my term of office. Dr Low was one of the most active agents in starting the scheme in 1923, and one of its most ardent supporters. This elaborate rostrum where I have been standing for the last two years, was a gift to the Society from Dr CARMICHAEL LOW.

During the war the Society lost touch with a large number of Fellows—some of these had died, others were prisoners of war while some had become our enemies. With the termination of hostilities active efforts were made to resume contact with lost Fellows and, after a sufficient waiting period, it was finally decided to remove from our roll all those who had died, and those of whom no news could be obtained. The result was a sudden fall in the numbers on our Roll from 1,688 in March, 1945 to 1,545 in March, 1946. You will see from the Annual Report that at the end of March, 1947 the number had risen to 1,646. During the past 2 months more Fellows have been elected, and with the candidates whose names I see on the Agenda of this evening's meeting, all of whom I trust will be elected, the grand total is now well over 1,720 almost a record figure in the Fellowship of the Society.

I think you will all agree that in spite of the many difficulties brought about by the war especially the restrictions placed upon paper to be used for publications, the TRANSACTIONS have maintained their high standard. Two very notable events in this connection may be mentioned. The first was the issue of the *Manson Centenary Number* containing a colour reproduction of Young Hunter's portrait which hangs in the Fellows room. We are indebted to Lady MANSION BAUER for the gift of this painting. The second event was the publication of the series of reports on the chemotherapy of malaria carried out by Dr HAMILTON FAIRLEY and his colleagues at Cairns in Australia. There is no need for me to stress the importance of this work which is well known to us all. The way it was carried out, and the manner in which it is reported, should serve as a model for all time as to how such an investigation by a team of workers should be carried out. The Society has indeed been fortunate in securing the publication of this work in its TRANSACTIONS. As I have remarked, there have been difficulties in publication owing to labour and the paper shortages, but recently restrictions have been relaxed with the result that, as the Treasurer has said, the last number published is the largest number of the TRANSACTIONS ever issued. The Society is much indebted to the many Fellows and others who have sent in papers for publication. There is a somewhat formidable accumulation of these, but we are now making every effort to get them to press as soon as possible. In this connection I may perhaps be excused if I ask those who send papers to the Society to make them as short as possible. When once a paper has been put together the author should go over it again very carefully to see if anything can be left out or if what has been said in two sentences cannot equally well be said in one. Furthermore, it appears that sometimes there is a tendency for authors to copy out their laboratory notes and present them in table form, when perhaps the whole table could be resolved into a single sentence. Tables were always very expensive to print, but in these days they are sometimes prohibitive. You will forgive me for making these suggestions, but a long experience with papers presented for publication has shown me that a little more care by the author before his paper

charts or drawings are presented, will be to his advantage in making early publication possible. During my two years of Presidency I have received the constant support of the Honorary Secretaries and the Treasurer. Dr HAMILTON FAIRLEY, who was Honorary Secretary with me before the war, continued in office though far away from home. Colonel DREW, now in Baghdad, acted for Dr FAIRLEY while he was away, and when you did me the honour of making me your President, Colonel DREW was made Honorary Secretary in my place. In 1946 Brigadier FAIRLEY returned and resumed his duties as Honorary Secretary. Soon after this Colonel DREW left on his appointment as Professor of Medicine in Baghdad. He resigned the Honorary Secretaryship which he had so ably filled, and Brigadier BOYD was elected in his place. To all these gentlemen, as well as to Dr MARRIOTT, the Treasurer, I owe my thanks. Dr MARRIOTT, I hear, is resigning the Treasurership, and I am sure you will wish me to thank him for his long years of service in this capacity. He has the advantage of having a foot in the City, and we always trust him when financial matters are under consideration. I hope we shall be just as fortunate when we elect the new Treasurer. I also, personally, and the Society as a whole, owe very much to the staff of the Secretary's office. The work of clearing up after the war has been very arduous, but it has been carried out with cheerfulness and efficiency, and I think I can say that the work has never been better done than it is now under the leadership of Miss HOPPER, who has been appointed as Secretary in place of my sister, whom you all know so well, and who has recently retired. Miss HOPPER, and those who are carrying on the good work under her, have the advantage of having worked for some time with my sister. I sometimes wonder whether the Fellows of the Society know what the Society owes to Miss WENYON, who for so many years has served it honourably and well. You may take it from me that she is a remarkable woman. (Applause) Some have even gone so far as to say she should have been a man. She is just as efficient and thorough whether she is poring over a paper for publication in the TRANSACTIONS, peering into the corners of Manson House to see if it is clean, interviewing callers, seeing to the very extensive correspondence which our long list of Fellows involves, examining the boilers to see if they really leak—even stoking the fires—or studying the finances of the Society to see if money can in any way be saved, or whether insurance societies are paying up properly, and, above all, making the Honorary Secretaries and the Treasurer carry out their respective duties. Everything has been done with an extraordinary efficiency, and it is not saying too much to state that the success of the Manson House scheme was largely due to her enterprise and understanding. It was a great blow to us all when she decided to retire last March, but I am glad to say, however, that she has been persuaded to continue her work on the TRANSACTIONS, for you may take it from me there is no one more competent to prepare papers for the press. Whether I can make any other claims to distinction or not, I can at least claim the honour of being her brother.

During my two years of office we have lost by death over forty Fellows, amongst whom were two Past Presidents, Professor J W W STEPHENS and Colonel S P JAMES and Professor SIMON FLEXNER, Honorary Fellow since 1921. We also received news of the death over 2 years ago, of two other Honorary Fellows—Professor JULIUS MANNABERG, of Vienna, and Professor BERNHARD NOCHT of Hamburg.

Recently Professor EUGENE PAVLOVSKY Professor of Zoology at the Academy of Sciences, Moscow and foremost parasitologist in the U.S.S.R., was elected an Honorary Fellow.

I must now proceed with the business of handing over this Badge and Chain of office to my successor Sir PHILIP MANSION BAHR. It is quite unnecessary for me to say anything by way of introduction of one so well known to us all. I am sure he will fill the office which I vacate with distinction and with pride for I see on the first link of this chain the name of his illustrious father-in-law Sir PATRICK MANSION the first President of this Society.

The retiring President then invested Sir PHILIP MANSION BAHR with the Badge and Chain of office, and inducted him to the chair.

The President (Sir Philip Manson-Bahr) Dr WENTON ladies and gentlemen, Fellows of the Society and friends, it is hardly necessary for me to say how honoured I feel at being elected to this august position in succession to my old friend, Dr WENTON who has brought me up in the way I should go for I feel that I am a child of this Society as I have been reared in it since 1909. My old friend, Dr CARMICHAEL LOW has also kept me in the strait and narrow path. I will, of course do the best within my power to promote the interests of the Society and give you as good a spin as I can for your money although I cannot expect to reach the standard of Dr WENTON whose scientific shoes I am not worthy to unlace. When I think of my predecessors, Sir PATRICK MANSION Dr CARMICHAEL LOW Colonel S. P. JAMES, Sir RICHARD CHRISTOPHERS—the last of whom we are glad to see here tonight—and of those others who have left us for another world, I feel very humble. I have had a little training for this position during the last year as President of the Medical Society of London. This has taught me a great deal which I hope to bring into practice over the next 2 years.

I shall now announce the name of the third Vice President, as I am permitted to do by the Laws of the Society. I should like to appoint my friend, Professor H. E. SHORRY whom you all know as my Vice-President.

I now come to the most important part of my new functions. It is the presentation of the medals. First of all the Manson Medals for the years 1941 1944 and 1947 two of which have been awarded in retrospect.

The Manson Medal for 1941 has been awarded to Professor EMILE BRUMPT. It had been hoped that his son, Dr LUCIEN BRUMPT would attend and accept the medal for his father but this he has not been able to do, and Dr WENTON will receive the medal on behalf of Professor BRUMPT.

To all of us here the name of Professor EMILE BRUMPT is a household word. He belongs properly to the Old Guard of Tropical Medicine, though he is equally at home with the new. He has adorned almost every page of tropical medicine for the last 45 years. Apart from his original researches into every aspect of medical zoology, he is best known for that compendious book of his, the "*Precis de Parasitologie*," which for many years has been the guide, philosopher and friend of every serious student. Indeed, it remains as bright and fresh today as when it first saw the light over 40 years ago.

Professor BRUMPT has given us a new term for our medical dictionary in venodiagnosis. He has been a wizard in perfecting methods of rearing insects, especially ticks, in the laboratory. One of his last and most important services has been the introduction of *Plasmodium gallinaceum*, the malaria parasite of fowls, to laboratory workers and which has thereby shed so much light on the life history of malaria parasites in general and their therapeutics in particular.

Professor BRUMPT has always declared himself an admirer and follower of Sir PATRICK MANSON, and it is therefore appropriate that he should be the recipient of the Manson Medal for 1941.

The medal was then handed to Dr WENYON, who said that Professor BRUMPT had written asking him to say that he had been most surprised and happy to receive the news of the award in March last. Professor BRUMPT had asked him to be kind enough to interpret to the members of the Society his thanks for the flattering distinction that had been accorded and to report how very sensible he was of the honour.

**The President** It is most fitting that the Manson Medal for 1944 should have been awarded to Sir RICKARD CHRISTOPHERS, a former President of this Society, one who has done so much to forward its interests and one who has trodden so closely in the footsteps of the namesake of this medal.

It is mainly for his devoted services to scientific medicine in India that the name of CHRISTOPHERS is best known. This name is always associated in our minds with that of our late beloved Presidents, Colonel S. P. JAMES and Professor J. W. W. STEPHENS, who were his close companions and colleagues for over many years.

Sir RICKARD's investigations into malaria in India are classic and his numerous and authoritative papers will constitute landmarks in the history of the disease for all time. His zeal and enthusiasm are electrical in their intensity at the present time, for he is still an active worker. His knowledge of the subject is stupendous and his contribution on this subject is generally acknowledged to be outstanding. We all know of his gentleness and humility. There is no one more deserving of the Manson Medal than Sir RICKARD, who has commenced his work in this field when he had begun.

The PRESIDENT then handed the medal to Sir RICKARD CHRISTOPHERS.

**Sir Richard Christophers** Mr President, ladies and gentlemen, to the worker in tropical medicine there is no higher honour than the Manson Medal of this Society and I am deeply conscious of the honour you have done me by conferring it upon me although it is somewhat embarrassing to find oneself on a list of names such as that of Sir RONALD Ross, and even more embarrassing when you have to say something about it. I looked up the TRANSACTIONS to see what those who had come before me had done about it, and I saw that some had said nothing at all. I thought that was getting out of it rather too easily and there are one or two things I should like to say. I suppose I am now one of the few who have seen the growth of tropical medicine, and especially the great developments in the study of malaria from the beginning. The enormous expansion of that has been a remarkable thing to witness. During that time I have come in contact with many workers in my own and other subjects, and I should like to say what great pleasure and profit and much else I have had from those contacts. Some of the workers I am speaking of are here with us tonight. Especially have I been associated with Professor STEPHENS and with Colonel JAMES, and it is a great sorrow to me that neither is present here tonight to see me get the medal. I am sure they would have been pleased. In this sort of swan-song I might also mention the Service to which I belong, the Indian Medical Service. That Service has given me almost a lifetime of unexampled freedom with great encouragement and always with consideration. Again I think I should like to say how much life and work in the tropics has meant to me. I would not exchange my experience of all the wonderful things I have seen for anything else on earth. Finally I would like to thank my proposer and seconder and also the Council of the Society for granting me the medal. It is not only the honour that it confers upon me, but it carries a very friendly feeling from the Fellows of the Society and Miss WENTON and is a great pleasure from that point of view.

**The President (Sir PHILIP MANSON BAKER)** The Manson Medal for 1947 has been awarded to our very much beloved former President, Dr C. M. WENTON.

Dr WENTON is the most distinguished protozoologist in the world. That has been abundantly recognised by the bestowal on him of the Theobald Smith Medal of our sister Society in the United States.

Honours have been showered upon him recently and rightly so, for Dr WENTON was one of MANSON'S first pupils—he was caught at an early age and was almost the first in this country to undertake protozoology as a special subject. We must all agree that the choice was a very wise one and that our first President, in picking out Dr WENTON spotted a winner. His "Protozoology" is a very great work and remains the standard book on this subject throughout the whole world.

There is hardly a protozoal disease which has not been advanced by his

work or on which he has not bestowed some original contribution Not only is he a great protozoologist, but his particularly wise and level-headed counsel is sought on all manner of subjects and those who, like myself, have watched him preside, as he has done for many years, over the destinies of this Society will recognize how much Manson House owes to him, for in actual fact he has been one of the chief architects and mainstays of the Society We know, too, that he has been always a loyal admirer of PATRICK MANSON, and therefore there is no one more deserving and fitted than he to receive the Manson Medal for 1947

Dr Wenyon (on receiving the Medal) I have said so much from this end of the room of late that I think the less I say the better at the present time I want to thank you, Sir, for presenting me the medal so graciously, and the Council for forwarding my name for it and giving me this high reward When I look at some of the holders of previous medals, such as Sir RICKARD CHRISTOPHERS, I think I had better be silent and sit down

The President The last medal to be bestowed is the Chalmers Gold Medal

Dr DAVID GARNETT DAVEY has been awarded the Chalmers Gold Medal for 1947 He is the youngest research worker to be the recipient of this medal and probably, too, in the comparatively short period he has been engaged on research he has accomplished the most fundamental and distinguished work

Dr DAVEY has the great advantage of having graduated as a zoologist, and his training, first in Cardiff, then in Cambridge, and later in Boston, U S A, has stood him in good stead in preparation for the great piece of research work for which fate had destined him We now know that it was to his clear thinking and courageous attitude in defining his goal that the new synthetic biguanides M 4430 and M 4888 (paludrine) were elaborated by his colleagues, F H S CURD and F L ROSE, in 1944 These three names will be for ever associated in this all-British venture Possibly history may acclaim the discovery of paludrine in the same breath as that of penicillin and their collaboration as close and distinguished as that of FLEMING and FLOREY It is clear that Britain still continues to produce scientific workers of the front rank and there is no cause for despondency when we have men like these to point the way to fundamental discovery It is therefore most appropriate that Dr DAVEY should be the recipient of the Chalmers Medal for 1947 and with this we must express the hope that he will long continue to contribute towards the ever-growing list of miracle drugs in the conquest of tropical disease

Dr Davey Mr President, ladies and gentlemen, I am deeply sensible of the honour the Society has done me tonight in awarding me the Chalmers



Medal. I do not want to make a long speech, but I should like to tell you how much I have benefited, in my work on malaria, from having such extremely able colleagues as Dr CURRIE and Dr ROSE, who were responsible for the synthetic chemical side of the work, and how much paludrine itself benefited from the work which Professor FAIRLEY did at Cairns. I doubt very much if the Chalmers Medal would have been given me tonight if I had not been fortunate enough to have had such colleagues.

I should have many years of life left in which to do research, and I hope I will be able to devote them to the service of at least some aspects of tropical medicine. I will try to make those years worthy of the Chalmers Medal and of the trust the Society has placed in me.

**The President** There are one or two words I would like to add as the new **PRESIDENT** that is to say how proud I feel at having these two distinguished gentlemen as Honorary Secretaries of this Society. I fully realize that we have two men here who have done outstanding work in the last few years in helping us in a great measure to win the second of the great wars of this century. Therefore I feel very humble in sitting in this place between two such stars one on the right, the other on the left. Possibly I feel, too, that they have not yet received the full reward of their great services to humanity.

This concludes the Annual General Meeting. It will now be succeeded by an Ordinary Meeting at which my friend, Brigadier SYDNEY SMITH, is going to show you some beautiful photographs taken in the course of the war and, as I have to leave to keep a long-standing engagement I will ask Dr WINTON to resume the Chair.

## ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1

on

Thursday, 19th June, 1947, at 8 45 p m

THE PAST PRESIDENT

Dr C M WENYON, C M G, C B E, F R S,  
in the Chair

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### PAPER AND LANTERN SLIDES

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## PRISONERS OF WAR CAMPS IN THE MIDDLE EAST AND THEIR MEDICAL PROBLEMS

By

Brigadier SYDNEY SMITH, F R C P, late R A M C  
(Précis of Paper)

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Brigadier SMITH first outlined the arrangements for the collection and accommodation of prisoners of war, and described the camps in which they were incarcerated, particularly the large base camps in Egypt. He emphasized the necessity for choosing the right type of personnel for the camp staff, and gave an account of the camp sanitation and of the methods adopted to keep the prisoners as fully occupied as possible. Many lantern slides were shown to illustrate the artistic talent of the Italian prisoners, who constructed, from the most primitive materials, numerous models and statues which adorned the open spaces of the camps, the high-light being a reproduction of Eros which occupied its traditional site in the "Piccadilly Circus" of one of the larger hospitals. So realistic were some of these models that a photograph of a tank, taken by Brigadier SMITH, was at first held back by the Censor because he considered that it might divulge important details relating to a secret weapon of war!

Brigadier SMITH then gave an account of the medical arrangements in the camps, and pointed out the danger which existed, particularly in the early days, of epidemics of typhus, enteric fever, and dysentery. After the camps were stabilized, each "cage" was under the charge of a medical officer and orderlies of the same nationality as the prisoners, who were responsible for dispensary treatment and for sending the more serious cases to the camp hospital which was commanded by a British medical officer assisted by a staff of prisoners. From the camp hospital patients requiring specialist treatment or nursing facilities were transferred either to an adjoining British General Hospital or to the P O W Section of a British General Hospital.

Despite the dangers inherent in the presence of these crowded and initially lousy communities, the general health of the inmates remained remarkably good and the spread of infectious diseases in anything like epidemic proportions was almost non-existent. Although there was a tendency to attribute many conditions which developed to dietetic deficiency there was, in fact, very little true deficiency disease. The diagnosis, however, was a favourite one with the prisoners themselves, as it entailed more liberal rations. The only significant outbreaks of deficiency disease were encountered among Libyan prisoners, who suffered from a moderate outbreak of scurvy shortly after their capture in 1941 resulting from dietary deficiencies prior to capture. In 1941 these same prisoners suffered from an outbreak of pellagra. Some 1 108 cases were notified, and of these 997 were mild, ninety-six were moderately severe, and thirteen were serious, one being fatal. The outbreak was controlled by modifying and adding to the standard diet scale—maize was eliminated, the bread, meat and vegetable ration was increased, and 1 oz. of yeast was given daily as long as supplies lasted—at a later date a small ration of ground nut (2 oz.) and red palm oil was added. In the winter of 1941-42, some 500 cases of glossitis occurred in the same camp the aetiology of which was uncertain at the time. A few months later there was an outbreak of so-called "night blindness," the bona fides of which were more than doubtful. The last epidemic of deficiency disease was a second explosive outbreak of glossitis, involving some 1 746 Libyans. This was carefully investigated and was attributed to a decrease of the intake of riboflavin from the bare daily minimum of 1.5 mg. to 1 mg. The cases failed to clear up on ascorbic acid but made a rapid recovery when milk, eggs and yeast were added to the ration. Six cases made a dramatic recovery on riboflavin. The interesting fact emerged that though this appeared to be an isolated example of true riboflavin deficiency none of the cases, when examined with the slit lamp, was found to have the marginal corneal vascularization associated with that condition.

Brigadier SMITH concluded by emphasizing the importance of the careful supervision of these prisoner-of-war camps, and the bearing which this had, not only on the health of the prisoners themselves, but on the surrounding military communities.

## COMMUNICATIONS.

AINHUM IN A LEPER  
BY  
ETIENNE MONTESTRU'C, M D  
AND  
PIERRE CAUBET, M D  
*Institut Pasteur de la Martinique*



In a case of leprosy we have encountered a lesion of the little finger which radiographically proved to be a case of ainhum. The observation is of interest in showing that the condition is not exclusively one of the toes, but that it may occur also in the fingers. The photographs show very clearly the tendinous retraction and the process of pathological amputation. It seems possible that leprosy may be one of the causes of ainhum, as it appears to be of perforating ulcer of the sole of the foot and gonodou. It is noteworthy that whether on an upper or a lower limb ainhum occurs on the fourth or, as is more usual on the fifth digit and

that this distribution is in the areas controlled by the ulnar or lateral popliteal (peroneal) nerves, which, as is well known, are these most usually affected by the leprosy bacillus



## MIXED DEFICIENCY DISEASES IN INDIA A CLINICAL DESCRIPTION

BY

R PASSMORE, D M , Capt I M S ,  
*Director, Nutrition Research Laboratories, Coonoor, South India*

During recent years there has been a marked change in our conception of deficiency diseases TROWELL (1946) has pointed out that in Africa the symptom complexes commonly described under this heading are not widely distributed in that continent, whereas the effects of inadequate food intake are far-flung A similar state of affairs exists in India Standard textbooks of medicine and tropical medicine for the last two generations have contained a section on deficiency diseases usually confined to accounts of beriberi, pellagra, scurvy, rickets and osteomalacia, a student of nutrition in India can derive only small assistance from such a section For he will soon observe that although direct and indirect evidence of ill health due to an insufficiency of food is widespread amongst the people of all parts of the country, yet the diseases named above have only a limited distribution Except in the northern districts of the Madras Presidency, beriberi is a rarity Pellagra is rare except in occasional outbreaks amongst the small maize-eating section of the population Scurvy is excessively rare, being usually only seen under famine conditions Rickets and osteomalacia are largely confined to the purdah population in North Indian cities In fact it is possible to have a wide experience of medicine and nutrition in India and to have seen none of these diseases Yet deficiency diseases are widespread In this paper an attempt is made to give a concise clinical description of the common signs and symptoms of mixed deficiency diseases, other than the five mentioned above, as they are present in large numbers amongst persons from all parts of India

The order of the description is based on the manifestations of deficiency diseases in the different systems of the body No detailed attempt is made to discuss the evidence, which assigns the cause of a particular sign or symptom to a deficiency of a single factor Separate accounts of specific deficiencies

are not given. In practice in India, it is rare for a single sign or symptom of malnutrition to be found alone. Inadequate food intakes of essential vitamins and minerals are frequently associated together with an insufficiency of total calories. In the resulting clinical picture the features of a single deficiency may predominate, owing to individual circumstances, but a more careful examination will usually reveal the effect of a general deficiency of the diet. It is essential to stress the uniformity rather than the diversity of the symptom complexes and to emphasize that, broadly speaking deficiency diseases fall within a single pattern, because in the important field of prevention and treatment attention must be paid primarily to both the quality and quantity of the food as a whole, rather than to deficiencies of specific factors. The use of vitamins and minerals in their pure state can only be an accessory form of treatment and can never be a substitute for a wholesome diet. To quote Sir JONIX MEGAW "we must be on our guard lest vitamins should become a craze."

No hitherto undescribed symptom complex and no new sign or feature of malnutrition are recorded in this paper. Rather it is an attempt to correlate our present knowledge and give a simple descriptive account, which it is hoped might prove useful to those responsible for medical education, both undergraduate and graduate, in the tropics. A great expansion of medical education is planned, both in India and in the Colonial Empire. It is essential that we think out and plan what is to be taught in these new schools and colleges.

A large literature containing accounts of particular clinical aspects of malnutrition as seen in India exists. The eye manifestations have been described by WRIGHT (1931 and 1934), DHURANDHAR and BOMAN BEHRA (1940) KIRWAN SEN and BISWAS (1941), KIRWAN SEN and BOSH (1944), RAJAGOPAL (1941), ATKROYD and VERMA (1942), VERMA (1942a and b) phrynodermis by RADHAKRISHNA RAO (1937a and b 1938) angular stomatitis by ATKROYD and KRISHNAN (1938 and 1938), ATKROYD and RAJAGOPAL (1936), and ATKROYD, KRISHNAN and PASEMORE (1939) the drogeental syndrome by NAIR (1939), KARUNAKARAN and NAIR (1940) and MITRA (1943) nutritional diarrhoea by ATKROYD and GOPALAN (1945). A general description is given by HEILIG (1943).

The following account is based on personal observations made before the war whilst on the staff of the Coonoor Nutrition Research Laboratories, and throughout the war on active service with Indian troops. At Coonoor frequent occasions arose for visiting and inspecting school children, for observing famine districts and famine relief work and for a study of estate labour. During the war exceptional opportunities were available at base hospitals for the observation and treatment of Sepoys, who had been evacuated from the eastern front on account of a nutritional breakdown, following the stress and strain of the Burma campaign.

## AETIOLOGY

A breakdown in nutrition can theoretically arise either from a defective intake of essential nutriments, an inability of the intestines to absorb them or a failure of the tissues to utilize them. In practice all these possibilities occur, sometimes in the same individual. In the tropics occasionally a patient with a long history and well-developed signs of malnutrition dies and at postmortem no evidence of any infectious or other disease is found. In such a case death is attributable to a failure to obtain the essential nutriments in the diet over a prolonged period of time. Alternatively, all the signs of malnutrition may be fully developed in a patient suffering from a chronic infectious disease, despite a satisfactory dietary intake. The physical signs of malnutrition can be fully and completely demonstrated in many cases of generalized tuberculosis, despite an adequate dietary history and with a satisfactory intake of protective foods. No assessment of nutrition either in a single patient or in a large group of persons is complete without a good dietetic history and an estimation of the nature and strength of the parasitic infections present.

In general it may be said that the principal causes of malnutrition in India are a deficient total food intake (inadequate calories), chronic malaria, a deficient consumption of milk and vegetables (inadequate amounts of vitamins A and the B<sub>2</sub> group) and chronic intestinal infection with protozoal and helminth parasites. There are, of course, numerous other factors which may be operative.

## DESCRIPTION

*General*—Two of the earliest and most prominent features of malnutrition are a loss of energy and a loss of weight. Listlessness, lack of enterprise and interestedness are important evidence of early malnutrition. An observer may readily detect these amongst groups of school children, labourers or soldiers. In the very young, a sign of inadequate nutrition is a failure to get into mischief. The listless attitudes and quiet demeanour of children, sitting patiently and uncomplaining around relief works and camps in famine areas without any spontaneous games or play, are characteristic manifestations of their poor nutrition. A few minutes' observation of the energy and initiative displayed by a group of labourers at work or a party of soldiers carrying out fatigues can provide valuable evidence for assessing their nutritional state. Severe cases of malnutrition in hospital often display a complete lack of interest in themselves and their surroundings. There may be insufficient initiative to attempt to eat and a total absence of any desire to gain in weight. The weighing machine is an invaluable guide to a schoolmaster in assuring himself that his children are receiving adequate amounts of food. It is equally useful to officers in charge of recruits' training depots or doctors responsible for an infant or



children a clinica. It is generally possible to get a good idea of the nutrition of a patient seen for the first time by picking up a fold of skin on the abdominal wall or over the pectoral muscles. The quantity of the subcutaneous fat, the laxity of the subcutaneous tissues and the underlying muscle tone can provide an indication as to whether there has been any recent loss of weight. In severe malnutrition as much as 40 or 50 lb. may be lost there is often an almost complete absence of subcutaneous fat and a great wasting of voluntary muscle. Emaciation may be so marked that patients can be aptly described as living skeletons.

Numerous attempts have been made in India and elsewhere to correlate weight and other physical measurements with nutritional status and various nutrition indices have been propounded in an attempt to put a numeral figure to an assessment. Experience has shown that these indices are of little value in diagnosis. A full account of such an attempt to correlate malnutrition with physical measurements in India with a review of the literature is given by ATKROYD, MADHAVA and RAJAGOPAL (1938).

Dehydration is a frequent concomitant of malnutrition. In many instances this is no doubt due to an inadequate water supply and difficulties involved in getting it. But this is not always so. The starving destitutes who roamed Calcutta during 1943 were frequently severely dehydrated. Yet Calcutta has an abundant water supply opportunities for drinking being available at almost every street corner. Patients with malnutrition in hospitals readily become dehydrated unless steps are taken to ensure an adequate fluid intake. The normal stimulus of thirst appears to be in abeyance. Oedema, presumably of nutritional origin, associated with low plasma proteins is seen not infrequently. It may be generalized, but is usually more extensive in the lower limbs. In severe cases the whole mechanism of the body for the regulation of water metabolism becomes disordered. On more than one occasion dehydration and oedema have been seen co-existent in different parts of the same individual. For this reason intravenous hydrotherapy which is frequently necessary must be carefully watched.

An apology is perhaps needed here for expounding the obvious. It may be argued that everyone knows that an ill nourished person loses weight, becomes thin, is easily tired and devoid of energy and initiative. However recent medical literature contains many descriptions of complex chemical and physiological tests, involving elaborate apparatus for assessing nutrition and has emphasized the difficulties and errors that may be met. As a result many doctors, ignorant of the principles involved in such tests and without the means of carrying them out, have lost confidence in themselves and fail to use the simple observational methods. They become afraid or unwilling to express an opinion on nutrition and are all too ready to summon the specialist. In India at least this is unfortunate. Practical nutritional assessment seldom requires laboratory facilities or expert knowledge. It has been found that a

competent opinion on nutritional status can in almost every instance be given as a result of simple clinical examination, in which the above elementary and commonsense observations play a large part

### THE SKIN

An early and frequent manifestation of defective nutrition of the skin is a proliferation of the cells of the epidermis giving rise to a superficial hyperkeratosis. There is usually some degree of atrophy of the sebaceous and sweat glands. As a result the skin becomes dry, rough and scaly. In slight cases only a small departure from the smooth, moist, velvety texture of normal skin may be apparent. Grades of increasing severity are found up to a very advanced condition, described as crazy-pavement skin by several writers (WILLIAMS, 1935, TROWELL, 1940, and PLATT, 1945). This condition is not uncommon in India and the name provides an excellent description of its appearance. Irregular cracks occur all over the dry hard epidermis. Most of these are superficial and are usually 2 or 3 mm apart, but quite irregular in pattern. Deeper cracks sometimes occur at wider intervals and these may be infected and ulcerated. The superficial dead layers of the squamous epithelium flake easily. There are usually small irregular areas of hypo- and hyper-pigmentation completely breaking up the normal uniformity of the skin and adding to the "crazy" appearance. All degrees of skin signs are more marked on the extensor surfaces of the limbs, but they may become universal. The face is usually least affected. Irregularity of pigment production is common. Symmetrical areas of increased pigmentation of the face are not infrequently seen. Hypo-pigmentation occasionally occurs. Unlike pellagra, the lesions are not photo-sensitive. Itching is characteristically absent. These very severe forms of crazy-pavement skin are only found in association with other manifestations of malnutrition.

In some cases the genitalia are especially involved. The scrotum becomes greatly thickened, dry and eczematous. On palpation it feels stiff and hard. A similar eczematous thickening of the vulvae may occur. In contrast to other nutritional skin lesions, there is much severe and troublesome itching. Secondary infection, mycotic, bacterial and parasitic, is common. Scabies was perhaps the most conspicuous clinical feature of the Bengal famine. The condition known as "tropical ulcer" is frequently associated with these changes in districts where the climate is moist and damp. The exact aetiology of these ulcers is *sub-judice*.

A distinct clinical type of hyperkeratosis occurs when the hair follicles are affected. The mouths of the follicles become filled with plugs of desquamated keratin, which project above the surface of the skin in the form of small papules about 1 mm in diameter. The papules are firm in consistency and do not pustulate. This condition, which is known as follicular hyperkeratosis, gives rise to a characteristic appearance and sensation to touch. In a well developed

case, on passing the finger tips along the outer surface of a child's arm, there is a characteristic sensation of roughness, as on the surface of a grater. The numerous little papules have been considered to resemble the skin of the toad and the condition has accordingly been called phrynodermis (NICHOLLS 1933). Again the extensor surfaces of the limbs are most affected, but the whole trunk may be involved. The face, genito-anal region, hands and feet are usually spared. The condition is much more frequently seen in children than in adults. It is not common in North India, but in certain districts in South India it is very prevalent. The condition is definitely associated with a poor dietary intake and there is a loose, but not very close, association with xerophthalmia. STANFORD (1945) has found that the condition occurs in England, where it is known under the name keratosis pilaris and is often present without any evidence of dietary defect. The exact causes of the disease remain undetermined. That it is often nutritional in origin in India is shown by the spectacular improvement which frequently follows vitamin A therapy. Its incidence has been widely used as a measure of assessment of nutrition in South Indian children and this has in practice been found reliable.

A second most important cutaneous manifestation of malnutrition is a peculiar degeneration that affects the junction of the skin and mucous membranes. All such junctions may be affected. The lips, the nostrils, the eyelids, the external auditory meatus, the prepuce of the penis, the vagina and the anus may all be involved. The lesion has essentially the same features at all sites. The mucous membrane over the junction becomes white, thickened and sodden and cracks appear. The white patches may extend inwards into the oral cavity over the surface of the glans penis or into the vagina, where a leucorrhoea may be set up. The cracks may be the seat of chronic infection and are then painful. The angles of the mouth is the site of election, which is always affected first and an associated glossitis is usually present. Hence the condition has been described as angular stomatitis. Cracking of the lips is a frequent and early feature and is known as cheilosis. The genital lesions are rarely if ever present alone and generally only occur in advanced cases. The combination of mouth lesions with eczematous thickening of the scrotum or vulva was first described in India by NAIK (1939) under the name orogenital syndrome. In rapid nutritional surveys in schools there is no necessity to inspect the genitalia, since the lesion if present will always be manifest round the mouth. Angular stomatitis is the commonest of deficiency diseases. Amongst a rice-eating population, it breaks out with remarkable regularity whenever the supply of fruits, vegetables, milk and meat falls. It disappears as soon as the intake of these protective foods rises. It can readily be cured by skimmed milk and yeast and hence is attributable to the lack of B<sub>1</sub> group of vitamins. Riboflavin often brings about marked improvement and the condition is undoubtedly closely associated with this vitamin but probably other factors in the B group play a subsidiary part.

The hair of the malnourished person lacks lustre and sheen, but no specific features such as the change in colour described as kwashiorkor in Africa by TROWELL (1945) and WILLIAMS (1945) has been seen.

The nomenclature of nutritional diseases of the skin is in some confusion. The following terms are suggested "hyperkeratosis (nutritional)," "hyperkeratosis follicularis," "crazy-pavement dermatosis," "eczema of the genitals (nutritional)," "angular stomatitis" and "cheilosis".

This list is drawn up more in accordance with common usage than with any sound system. Thus the condition described as "hyperkeratosis follicularis," and known to medical men throughout the tropics under this name, has been called by European dermatologists "keratosis pilaris". As the condition is far more widely known under the name hyperkeratosis follicularis, it would seem best to retain it. This name is, however, already in use amongst dermatologists, for a rare condition of doubtful aetiology, also known as "Darier's disease". This disease might continue to be called "hyperkeratosis follicularis (Darier)". The term "phrynoderma" or "toad-skin" has also been introduced and is widely used. The skin in these cases is always hard and dry. This term is not very accurate and is now redundant. "Crazy-pavement" skin is so descriptive of the condition that it is hoped that the term will be given the dignity of an official diagnosis without latinization. "Angular stomatitis" was originally introduced by STANNUS, who recently (1944) has pointed out its inadequacies as a descriptive term. Nevertheless, this name is now so widely used and its meaning generally understood that it would be undesirable to change it.

#### THE EYES

Xerophthalmia or keratomalacia is probably the most frequent sign of malnutrition which is seen in India. The description given by WRIGHT (1934) cannot be improved upon.

"The earliest manifestation of keratomalacia is night-blindness. The next evidence of this progressive deficiency is a peculiar smokiness of the conjunctiva, this is the earliest obvious sign just as the night-blindness is the earliest symptom. One may meet either or both of these in a youth who is apparently healthy looking in other respects. They are, however, danger signals. When the early signs and symptoms appear, if nothing is done, to augment the amount of essential food factors in the diet, the case rapidly progresses and marasmus sets in. *Pari passu* with the wasting there is an advance in the eye symptoms. As a rule, the smoky conjunctiva becomes dry and wrinkled, greasy looking, the cornea becomes dull and lustreless and eventually opaque. Later the cornea undergoes necrosis and ulceration and, if untreated, the ulcer perforates and the eye is ultimately lost."

The condition is frequently associated with Bitot's spots. These are glistening white plaques of dead conjunctival epithelium usually triangular in shape and firmly adherent to the underlying conjunctiva. They are generally bilateral and are surrounded by a dense brown pigmentation.

Early xerophthalmia is common in the rice-eating areas of India in parts of the Madras Presidency as many as 30 per cent of the children have been found affected. Although in the great majority of children the condition is arrested before serious defects of vision arise, yet in many cases the disease

progresses. All authorities are agreed that many thousands of persons in India are permanently blind from this cause. WRIGHT (1931) from his experience at the Government Ophthalmic Hospital, Madras, considers it the most important cause of blindness in childhood and youth.

The early signs of xerophthalmus, dryness, increased wrinkling and pigmentation of the conjunctiva and the associated night-blindness are undoubtedly closely related to dietary defects especially an inadequate intake of vitamin A, in children. In adults the interpretation is more difficult. They are not infrequently seen in persons, who are living on adequate diets, are unassociated with any visual defects and are not necessarily progressive lesions. In some cases they may be residual scars of an old lesion, which has been inactive for many years. In others it would appear possible that repeated attacks of conjunctivitis may be responsible. War experience has shown that, although vitamin A deficiency undoubtedly leads to night-blindness, by no means all night blindness is due to dietary causes and many other factors, including psychological strain, may be responsible. Nutritional night blindness responds promptly to vitamin A therapy.

In recent years two other eye manifestations of malnutrition have been reported in many parts of the world. The first is a superficial keratitis the patients complain of burning sensations in the eyes, mistiness of vision and photophobia. Various degrees of circumcorneal injection and corneal vascularization can be seen in most cases. Small corneal opacities and superficial ulcers are sometimes present. A slit lamp is necessary for detecting minor degrees of vascularization. The differential diagnosis from simple congestion or even interstitial keratitis is difficult for the inexperienced. The condition is almost invariably associated with cutaneous and alimentary signs of malnutrition and sometimes responds promptly to riboflavin therapy. The second is a defect in vision amounting in some cases to total blindness which may or may not be associated with pallor of the optic discs and optic atrophy and other nervous degenerations. This nutritional amblyopia usually follows upon long periods on deficient diets (many of the cases described have been prisoners) and is often associated with other signs of malnutrition. There is a steady improvement in vision in most cases when the patient is put on a good diet, and both vitamin A and vitamin B concentrates have been reported to accelerate recovery. In the writer's experience these two conditions are not commonly associated with severe malnutrition, as observed in general hospitals in India, nor do they appear common in Bengal (LUXWAN SEN and BOSE, 1944). That they are not infrequent in the South is shown by the records of the Government Ophthalmic Hospital Madras, where both have been extensively studied. (AYERST and VERMA, 1942 VERMA, 1942a and b.)

#### THE DIGESTIVE SYSTEM.

As already stated, loss of appetite is a prominent early feature of malnutrition this is usually primary but may be due to a fear of pain in the mouth

on eating or of flatulence and abdominal discomfort following food. These symptoms are common. The pain in the mouth is ascribable to a glossitis. The tongue is frequently swollen and enlarged, occasionally to such an extent that it is continuously pressed against the lower jaw and well marked dental impressions are visible. The organ is characteristically red. The mucous membrane may be seen to be desquamating in patches leaving a red raw surface visible. The papillae are often unusually prominent. Deep irregular fissuring is a common feature. Occasionally the tongue is seen to be small with a smooth atrophic mucous membrane with fine fissuring. Irregular patches of dark pigmentation are frequently present.

Severe dental sepsis is often seen associated with malnutrition. In advanced cases the abdomen is shrunken and scaphoid. Sometimes it is distended and tympanitic. Gurgling noises are common. "Pete men *gur gur jata hai*" is a frequent complaint.

Test meals show no characteristic features, but hypochlorhydria is common.

Diarrhoea is a most frequent and important symptom. It is rarely so severe as in the bacillary dysenteries. Six to eight times a day is the usual number of motions. The diarrhoea is always worse in the mornings and the bowels may give no trouble after mid day. Sometimes the intake of even small quantities of food sets off an evacuation. The stools are usually watery, faecal in colour and often contain visible particles of food, completely undigested. Microscopically undigested starch grains, vegetable fibres, etc., can be readily seen. Inflammatory exudates are characteristically absent. Excess fat is not often visible. The large, pale, fatty, offensive stool described as characteristic of sprue in Europeans is only rarely seen in Indians. The nature of the diarrhoea is such that it would appear to be the result of a "small-intestinal" hurry rather than any inflammatory process. Although the diarrhoea can usually be controlled temporarily by sulphonamides, especially sulphaguanidine, and sometimes by nicotinic acid, relapses are frequent. It is usually the most striking and important feature of severe malnutrition, associated with great wasting, "crazy-pavement" skin and the appearance of a living skeleton. It is generally the principal cause of death.

At postmortem a general atrophy of the whole intestine is usually seen extending throughout from the mouth to the rectum. All coats, mucosa, submucosa and muscle, are involved. The small intestine is the most severely affected, becoming progressively thinner from the duodenum down to the ileo-caecal junction. Indeed the last 2 feet are so thin and transparent as to resemble the texture of tissue-paper. The mucous membrane of the small intestine is smooth and atrophic and clearly its digestive and absorptive functions must be seriously impaired. Ulceration of any part of the gut is uncommon. The intestines of Sepoys, who had died with chronic diarrhoea and malnutrition after evacuation from the Burma battle-front, presented post-mortem an appearance identical with the intestines of monkeys, who had died

in a similar emaciated condition in the Coonoor laboratories, following the consumption for many weeks of a diet consisting of rice with minimal amounts of protective foods. The same gross atrophy of the small intestine was present in the experimental animals. A detailed pathological account with a full histological report of these monkeys has been given by RADHAKRISHNA RAO (1942). The following is an extract of his report —

Degenerative changes were constantly present in Auerbach plexus. In most cases the plexus was enlarged and oedematous, and hence easily recognized in the sections. Varying degrees of degenerative changes from cloudy swelling to complete degeneration were found in the ganglion cells. The number of ganglion cells involved in the degenerative process and their distribution in the plexus showed great variation in the several specimens. In chronic cases, normal ganglion cells were rarely seen and the plexus was represented by empty spaces containing skeletons of dead and degenerating ganglion cells, round cells, fibroblasts and glial cells.

The opportunities available for histological study of the human material have been very limited, but these findings have been confirmed in every case examined. All modern physiological research emphasizes the importance of the autonomic nervous system in controlling the functions of the alimentary canal. An early degeneration of the neurons in this system might well be responsible for a complete breakdown of the digestive processes, severely limiting the absorption of nutrients and leading to malignant malnutrition, refractory to treatment. Such a degeneration may well be an initial pathological event in a vicious circle of defective food intake, intestinal atrophy defective adsorption, further atrophy leading to the "tissue paper" small intestine and progressive emaciation.

Very little is known about the functions of the liver in cases of malnutrition in India. This is partially attributable to the difficulties in obtaining satisfactory postmortems. It may be said that cirrhosis at all ages certainly and primary carcinoma possibly are more common than in Europe. What relation these conditions have to nutrition is not known. In fatal cases of severe malnutrition the liver is usually small and invariably fatty.

#### THE CARDIOVASCULAR SYSTEM.

In minor degrees of malnutrition there are no clear-cut signs of cardiovascular disease. In more advanced cases there is a characteristic fall in blood pressure, which may be very low. A systolic pressure of 70 mm and a diastolic pressure which is unreadable are sometimes found. These low levels are always associated with other evidence of severe malnutrition and carry a poor prognosis. Cardiac failure with oedema is sometimes a terminal event. Ordinary cardiac stimulants are valueless and cases often end fatally after dragging on for many weeks. At postmortem the whole heart is found to be very small with marked atrophy of the muscle.

The hypotension, emaciation and pigmentation may present a picture strikingly resembling Addison's disease. However no improvement has

followed in a few cases to whom cortical extracts were given. The adrenal glands are usually small and atrophic, but present no specific changes.

### THE NERVOUS SYSTEM

A mild peripheral neuritis is occasionally seen associated with chronic diarrhoea and severe wasting. The disease is usually confined to the legs, where there is weakness and difficulty in walking. There is usually some muscular tenderness and the tendon jerks are sluggish. Occasionally the knee jerks are exaggerated. The condition is, in fact, secondary beriberi. Outside the endemic areas of beriberi, it is uncommon and is seldom the primary complaint of the patient. Recovery usually takes place slowly with the cessation of diarrhoea and *pari passu* with the gain in weight and general well-being. It does not appear to be accelerated by vitamin B, preparations either by injection or by mouth.

A nutritional amblyopia has already been referred to. In returned prisoners of war, neurological signs referable to the spinal cord lesions have been found in association with amblyopia. In a report (*Neurological Conditions resulting from Captivity*, November, 1945), the Consultant Neurologist, India Command, Brigadier DENNY BROWN, gives an account of fifty-six cases of spinal ataxia found amongst Indians, who had all been prisoners in Japanese hands for at least 2 years. There can be little doubt that these cases were nutritional in origin. No account of this condition in civil practice has been seen and it is probably rare. Subacute combined degeneration of the cord is uncommon in Indians.

The severe depression often associated with malnutrition in India has already been described. It is the main psychological feature. Manic symptoms and dementia, important accompaniments of pellagra, are rarely seen.

### THE BLOOD

Some degree of anaemia is usual in malnutrition. A haemoglobin of 7 to 10 grammes per cent is common. The haematological picture is very varied and inconstant, but a macrocytosis is usually present. However, very severe and even fatal cases have been seen in which normal haemoglobin levels have been obtained. Surveys on tea estates have indicated that the poorest rice diets contain enough haematopoietic factors to make good normal losses of haemoglobin. There is, however, no reserve and, when extra demands for haemoglobin arise as a result of malaria or ankylostome infection, these cannot be met and anaemia results. A full account of the severe tropical macrocytic anaemias, which were seen amongst Sepoys during the war is given elsewhere (PASSMORE, 1944). The aetiology of these anaemias remains obscure, but they are associated both with chronic malarial infection and with a deficient food intake, probably more closely with the former.



## TREATMENT

This section is confined to an account of the methods available for the treatment of patients who have been sufficiently ill to merit admission to hospital. No reference will be made to the public health problems of prevention or to the treatment in the field of the innumerable minor cases, who do not require hospitalization or for whom hospital beds are not available. These great problems in India are essentially economic and agricultural tasks and such medical advice as can be given is in practice limited by these factors.

### *General*

Severe malnutrition is always associated with mental depression and retardation. Many patients appear at first to have no desire to get better or even to live and parenteral treatment may have to be the first step. Other treatment may be of no avail, unless the co-operation of the patient is obtained and until he can be persuaded not only that it is a good thing to get better but also that it is possible for him to recover. To secure a right psychological attitude is the first essential of treatment. The patient's confidence must be won. He must be made to realize that the circumstances, which reduced him to his present condition, are now past. An energetic and persuasive ward sister can work wonders and is sometimes the most important single factor in recovery. Attractively served food, a bright and cheerful ward with ample decorations, a willing ward staff who are prepared to spend time and patience coaxing refractory patients to eat, these are the important elements of treatment and many sisters have been able to achieve much along such lines with extremely limited material. A cheerful convalescent patient, who has himself recovered from severe malnutrition, may prove invaluable in encouraging and helping bad cases. An active diversional therapy department is a most valuable asset.

### *Dietetic*

Elaborate and rigid dietary scales should be avoided. As far as possible each patient should be considered separately and his whims and fancies pandered to. They are often a better therapeutic guide than the physician's dietetic theories and should always be respected. It is important that feeds should be frequent and small. Some nourishment should be taken at least six times a day. It is generally convenient to have three main meals a day and to give milk drinks, suitable fruits, fruit drinks, sweets and sweet meats in the intervals. At first the main meals should be small, especially if diarrhoea is a prominent feature. Overloading the stomach will excite an over-sensitive gastro-colic reflex, cause flatulence and abdominal distress. Details of diets will not be given. These depend on the customs and taste of the people and the available food. In the past too much attention has probably been placed on the exact ratio of protein, fat and carbohydrate in the diet. The malnourished person usually has an aversion to fat and this should be

limited. Otherwise it may be said that any wholesome well-cooked food is valuable in small quantities. Pulses and coarse fruits are usually poorly tolerated and can often be seen passed completely undigested into the bed-pan. Meat and flesh foods are valuable and every effort should be made to encourage patients to eat them. However, they are not essential and need not be pressed against strong religious conviction. Milk and milk preparations are usually the sheet anchor of treatment. Curds are particularly valuable and are relished by all Indian patients. Tinned milk makes excellent curds.

Patients, who are first seen in a badly collapsed state, should not be given solid foods. Many fatalities have occurred during famines through sudden overloading of the stomach. Such patients should receive only a fluid diet for 48 hours or until their condition improves. Small quantities of diluted, citrated milk or sweetened fruit juice should be given hourly. Later an egg beaten up in milk may be added. For very weak patients unable to feed themselves continuous intragastric drip feeding, which may be carried on for many hours is convenient and a great saving of labour for the nursing staff. Dehydration may have to be overcome by the intravenous infusion of salines to which glucose may be added. For the treatment of hypoproteinaemia, concentrated solutions of both serum proteins and protein hydrolysates have been used. KRISHNAN, NARAYANAN and SANKARAN (1944) gave over 3,000 intravenous injections of a papain digest of meat to about 1,000 cases of starving destitutes in Calcutta and believe that this was responsible for the saving of some lives, but the conditions of work made accurate assessment of its value impossible. LOWE (1946) stated that, in the experience of the School of Tropical Medicine at Calcutta, the results of intravenous therapy with proteins and protein products were on the whole disappointing. The practical value of this form of treatment in India is clearly still *sub judice*. In the treatment of nutritional oedema associated with hypoproteinaemia, mercurial diuretics have been found to be of value by numerous physicians.

### *Vitamin therapy*

Vitamin A preparations are valuable, especially in children. The various skin conditions previously described are nearly always improved by vitamin A. Sometimes spectacular successes are rapidly obtained, more often improvement is slow and follows only upon several weeks of treatment. Night-blindness is often rapidly relieved. A single dose of 200,000 i.u. will usually produce improvement in a few hours. The signs of keratomalacia improve more slowly. There is usually a general improvement in the health and well-being of the child. In adults the value of vitamin A (aneurin) therapy is much less.

Vitamin B<sub>1</sub> (thiamin) has been given both by mouth and subcutaneously to many patients with peripheral neuritis and an associated malnutrition. No evidence has been obtained that the vitamin has any effect on the rate of recovery from the neuritis.

Nicotinic acid will often give spectacular results in relieving the pain associated with glossitis and stomatitis. New mucous membrane is rapidly formed over the tongue and the pain present during chewing and swallowing diminished. Nutritional diarrhoea is also rapidly controlled in many cases by nicotinic acid. A dose of 100 mg three times a day by mouth is generally adequate, but some cases only respond when the dose is given by subcutaneous injection. If any benefit is going to occur signs of improvement are usually manifest within 48 hours. In the absence of signs of improvement it is valueless, persist with the vitamin longer. Although dramatic relief of symptoms is frequently obtained, in other cases the stomatitis and diarrhoea appear completely unaffected by the vitamin. It would appear at present impossible to forecast, by clinical examination, which cases are likely to improve and in which the symptoms are likely to remain unaffected. In my experience not more than 50 per cent. of patients are benefited. Although rapid relief often occurs, complete cure is seldom achieved by nicotinic acid alone, unless an all-round improvement in the diet is simultaneously effected and relapse is frequent if this is not maintained. To sum up it may be said that nicotinic acid is a valuable, but uncertain therapeutic weapon.

Riboflavin has not been available in India hitherto for therapeutic purposes except on a small scale. It has been shown to be effective in relieving the signs and symptoms of superficial keratitis. Some cases of sore mouth with excoriations at the angles and with other manifestations of muco-cutaneous degeneration have responded promptly to riboflavin. A dose of 5 to 10 mg daily by mouth or subcutaneously is usually adequate. In general, the comments made on nicotinic acid therapy apply also to riboflavin.

Although, as already mentioned, clinical evidence of scurvy is very rare in India, most cases of malnutrition have been living on diets containing barely minimum amounts of vitamin C. There is therefore every indication for providing liberal quantities of vitamin C. This can best be done by giving fruit or fruit juices rich in the vitamin. If these are not available, ascorbic acid (50 to 100 mg daily) should be given. Sprouted grains and pulses are also a valuable source of the vitamin.

Fresh yeast, dried yeast and yeast preparations such as marmite and rege-mite have proved valuable in the treatment of malnutrition. The best results have been obtained treating the minor manifestations, especially stomatitis, in the field. Undernourished children are frequently benefited. Results with severe cases of malnutrition in hospital have been disappointing. If an adequate supply of protective foods is available in the diet, supplementing the normal source with the concentrates in yeast appears to be of little value. Perhaps the patient is unable to absorb or utilize the extra vitamins. Yeast and its preparations are particularly distasteful to Indians and it is now the writer's custom to prescribe them only when a hospital dietary is not completely satisfactory. In India it is probable that yeast therapy will have its maximum beneficial effect in out-patient clinics and field treatment.

In recent years vitamin therapy has been extensively used in the treatment of malnutrition. Pure vitamins have been given both parenterally and by mouth and many preparations of vitamin concentrates have been employed. Although vitamin therapy is valuable and has its place in the treatment of malnutrition, results are on the whole disappointing. In tropical practice the slow and uncertain improvement, following the exhibition of pure chemicals to patients with signs of malnutrition, contrasts strongly with the prompt and spectacular results usually obtained from the use of the appropriate chemotherapeutic remedy in acute infections. Vitamin therapy can only be supplementary and can never, under any circumstance, satisfactorily replace a defective supply of food.

#### TREATMENT OF CARCINOMA INFECTIONS

It cannot be too strongly stressed that no dietetic treatment of the malnourished can be effective in the presence of infections. An essential part of the treatment of malnutrition consists in a diligent search for protozoal and helminth parasites and in their eradication. Experience has shown that protozoal diagnosis is often difficult in the under-nourished. Although a patient search may have failed to find malarial parasites and amoebae, in some cases prompt improvement follows anti-malarial and anti-amoebic treatment. The presence of persistent unexplained anaemia or diarrhoea is an indication for the use of these remedies, even in the absence of an exact diagnosis. The sulphonamides, especially sulphaguanidine, are generally effective in controlling nutritional diarrhoea, although the effect is usually temporary and relapse frequent. In emaciated persons very small doses are often adequate.

Following severe malnutrition recovery is always slow. Even under the best hospital conditions there is always a definite mortality. In advanced cases it would appear that irreversible degenerative processes have taken place. Convalescence must never be hurried and after severe illness activities must be limited. Unexplained sudden deaths have occurred in patients who appeared fully convalescent and who were up and about in their wards. Many patients can never regain their full health and relapses are frequent if the patients are again subjected to nutritional strains. Soldiers, who have been evacuated to base hospitals on account of nutritional breakdowns, should never be sent on active service again.

#### DISCUSSION

A description of malnutrition in India cannot be complete without brief references to its relationship to the conditions frequently described under the headings pellagra, sprue, ariboflavinosis and to the excellent descriptions of deficiency diseases in Africa.

Many of the features of malnutrition seen in India are common to pellagra. In particular the glossitis and diarrhoea appear indistinguishable in the two conditions. In addition, the skin changes bear some resemblance, especially

as the hyperpigmentation and hyperkeratosis, which are commonly seen in deficiency states in India, are usually more severe on the exposed surfaces. But the extreme photo-sensitiveness, characteristic of pellagra and the extensive skin lesions are not seen. STANFUS (1938) considered that similar lesions in Africa were pre-pellagrous in nature and observations in India lent some support to this view. Nevertheless, although glossitis and nutritional diarrhoea are common and often so severe in rice eaters as to be responsible for many deaths, yet true pellagra is very rare. That it can occur must be accepted. Examination of photos of cases observed by RAVAN (1940) in rice eaters at Vizagapatnam leaves no room for doubt that they are pellagra. However, numerous cases, which have been shown to the writer in various parts of India, diagnosed as pellagra, have borne little resemblance to the florid cases which are seen in *epiderma* in Egypt. The extreme and characteristic photo-sensitivity seems to appear only in maize eaters. Why the poor rice eater who suffers from nicotinic acid and other B deficiencies, so rarely gets true pellagra is unknown. The problem has been discussed by ARKROYD and SWAMINATHAN (1940), who have shown that it cannot be explained by differences in the content of known food factors in the two cereals. Pellagra is not simply a nicotinic acid deficiency. Maize must differ from rice, either in the lack of a hitherto unrecognized dietary factor or in the possession of a toxic component with the property of photo-sensitizing the skin. True pellagra occurs amongst maize eaters in India, and BAJAJ (1939) has given an account of the disease in the Kangra valley.

A condition clearly very similar if not identical, to the picture of malnutrition which has been described, is the disease known as sprue. This is a diagnosis not uncommonly applied in India to European and Anglo-Indian patients, but rarely to Indians. The features usually ascribed to sprue are those of a wasting disease, associated with glossitis, macrocytic anaemia and diarrhoea with the passage of pale, large, frothy offensive stools. Only in the last respect does it differ from the picture of malnutrition as usually seen in India. Otherwise it complies in detail with the general description of multiple deficiency diseases. The character of the stool is by no means an absolute racial distinction. The typical sprue stool is occasionally seen in an Indian and sometimes absent from an otherwise typical European case. The nature of the stool probably depends more on the quality of the diet and the type of intestinal flora than on the pathology of the disease. There can be no question that sprue is a mixed deficiency and that the general clinical picture is the same as that of malnutrition in Indians. The difficult problem is the nature of its aetiology and epidemiology. In Europeans the disease is only occasionally associated with a defective food intake and in some instances there is epidemiological evidence suggesting an infective agent. The introduction of the linguistically mixed term *para-sprue*, which is recently becoming popular as a label for diarrhoea of nutritional origin in Indians, seems an unnecessary complication of nomenclature.

The diagnosis of ariboflavinosis or hyporiboflavinosis, first introduced in America, is now becoming widely used. The condition has been fully reviewed by STANNUS (1944). Many signs such as angular stomatitis, cheilosis, generalized mucocutaneous degenerations, scrotal eczema, superficial keratitis have been included by different workers, based on therapeutic response to riboflavin. Experience in India has shown that all these conditions do sometimes respond dramatically to riboflavin, but that the effect is erratic and inconstant. Further complete cure is seldom obtained without a generalized dietary improvement, and relapses are frequent. Riboflavin deficiency can only be a part of the aetiology of these diseases. A pure riboflavin deficiency probably never arises under natural conditions. It would seem better to preserve the older descriptive diagnoses rather than to introduce a new term involving an incomplete description of the aetiology of the conditions.

In a review of malnutrition in Africa, TROWELL (1946) makes the following assertion —

“The truth is that the pattern of malnutritional disease in the African is different from that seen in other parts of the world, and until this is recognized malnutrition will not be diagnosed in Africa. A new disease is in its labour pains, probably it is a pattern of associated diseases.”

With the second sentence it is possible to be in full agreement, but experience in India does not provide evidence to support the first. Except in minor details, TROWELL's descriptions of deficiency disease in Africans apply with equal accuracy to the disease in Indians. It is possible that accounts of deficiency states in India and elsewhere have been misleading because for the most part they have been limited to descriptions of lesions in one system of the body only or to attempts to describe the picture of a deficiency resulting from the lack of a single dietary factor. The medical literature from Africa has by contrast more frequently emphasized the description of the patient as a whole. For this reason a better description has probably been given. The picture of malnutrition may vary in detail from person to person and in different parts of India. Every degree of severity from a minor sign unnoticed by the patient, to a severe irrecoverable disease, may readily be seen. But all the signs appear to fall within one broad outline, within which a great variety of detail may occur. It is not the present purpose to review the numerous accounts of deficiency states as ascribed in the African and in other races of mankind. But the manifestations of malnutrition in the Indian described in this paper would appear to differ in no important fundamental from descriptions in other races coming from observers in different countries. A limited experience of deficiency diseases amongst Africans in Eritrea, Europeans in India with sprue, Chinese refugees in the Far East, and Japanese prisoners from the Burma campaign, has produced no evidence to suggest that there is any important clinical distinction between the manifestations of these diseases in different races.

## SUMMARY

A description of the signs and symptoms of deficiency disease in India is given. The general picture is similar to that of nutritional diseases in Africa and other countries.

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# • ENZYME-PURIFICATION OF POLYVALENT ANTIVENENE AGAINST SOUTHERN AND EQUATORIAL AFRICAN COLUBRINE AND VIPERINE VENOMS

BY

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Since the date of preparation of antivenene at the South African Institute for Medical Research, continued efforts have been made to improve the therapeutic properties of the product.

Venoms detoxicated with formalin proved to be safe and efficient antigens (GRASSET and ZOUTENDYK, 1932, for detail see GRASSET, 1945). *Naja flava* and *Bitis arietans* venoms have, immunologically, the highest and widest antigenic powers among the representatives of their groups (GRASSET, ZOUTENDYK and SCHAAFSMA, 1935). The observations of GRASSET and ZOUTENDYK (1938) showed that *B. arietans* antivenene has but limited effect on *B. gabonica* venom, owing to the coagulant fraction in the latter, and a special polyvalent antivenene was therefore produced, including the venom of *B. gabonica* as an antigen.

Fractional sodium sulphate precipitation removes the larger proportion of inactive serum proteins, the antibodies, contained in the pseudoglobulins, were concentrated three to four times (GRASSET, 1932). Serum reactions due



to this product are not rare and for this reason, enzyme-purification (PARFENTJEV 1936 POPE, 1939) was adopted, using POPE's technique. This method of pepsin digestion has been applied to polyvalent antivenene prepared against the venoms of the South African *N. flavus* and *B. aristatus* and to antivenene prepared against the venoms of *N. flavus*, *B. aristatus* and the Equatorial African *B. gabonica*.

The finished refined product is crystal clear almost colourless, and of fairly low viscosity the protein content is adjusted to from 10 to 16 per cent. before ampouling. It is free from residual active pepsin and is stable in the ice chest and at 15° to 25° C.

Rabbits of from 1,500 to 2,000 grammes, which received from 1 to 5 c.c. of undiluted antivenene, either intravenously or intramuscularly remained normal during the 30 days of observation. Mice of 20 grammes, receiving 1 c.c. subcutaneously or 0.25 c.c. intravenously remained normal also.

Comparative data for a batch of *N. flavus*-*B. aristatus* antivenene (Serum I) and a batch of *N. flavus*-*B. aristatus*-*B. gabonica* antivenene (Serum II), regarding neutralising titres before and after purification, rate of concentration, yield and nitrogen content are shown in Table I. All titrations recorded in this table, as well as in the following table, were done on rabbits (1,800 to 2,000 grammes). The raw sera were titrated in volumes of 3 c.c. against increasing amounts of venom, and the purified sera in volumes of 1 c.c. All titres in Table I are "corrected," i.e., approximately 4/5 of an m.l.d. is subtracted from the amount of venom neutralized *in vivo*. The corrected titres of the raw sera are

TABLE I.

COMPARATIVE PROGRESS FOR TWO BATCHES OF ANTIVENERE, BEFORE AND AFTER CONCENTRATION

	Vol. in c.c.	mg. N in 1 c.c.	2lg. venom neutralized by 1 c.c. serum.			Rate of concentration of antibodies.			% of recovered antibody against		
			N flavus	B. aristatus	B. gabon.	N flavus	B. aristatus	B. gabon.	N flavus	B. aristatus	B. gabon.
Serum I											
Before concentration	4.84	13.7	0.87	0.2	—	—	—	—	—	—	—
After concentration	800	25	2.7	23.8	—	4.7	3.0	—	23	42	—
Serum II											
Before concentration	6.000	14.4	0.97	4.9	3.0	—	—	—	—	—	—
After concentration	880	30.7	1.4	22.6	18.4	0	4.0	4.4	25	4	44

further divided by three, to give the neutralizing value of 1 c c. It is assumed that the natural resistance of the animal accounts for the neutralization of approximately 4/5 m l d, and this "correction" was introduced to minimize the error in judging the results of titrations done on a very low toxin level.

The nitrogen determinations were done colorimetrically.

It will be seen that the titres of antibodies against *N flava*, *B arietans* and *B gabonica* have increased from four to six times, the concentration of protein nitrogen being approximately doubled, i.e., an increase in the antibody/nitrogen ratio from two to three times, as an expression of the increased purity. The recovery of *N flava* antibodies is approximately 50 per cent. The recovery of *B arietans* and *B gabonica* antibodies is slightly lower.

### GROUP NEUTRALIZING PROPERTIES OF ENZYME-PURIFIED ANTIVENENE

In Table II are given the "uncorrected" titres of 1 c c of Serum I and Serum II, after concentration, against the specific venoms, and against various other Southern African and Equatorial African snake venoms. The minimum lethal doses (death in 2 to 4 hours) in mg of venom are given for rabbits of the weight used in the titrations. Identical samples of venom were used in the titrations and for the determination of the lethal doses.

TABLE II

TITRES OF SERUM I AND SERUM II AGAINST VARIOUS SNAKE VENOMS. THE TITRES ARE GIVEN IN MG VENOM NEUTRALIZED BY 1 C C OF CONCENTRATED SERUM. THE M L D OF THE VENOMS IN MG.

	<i>Naja flava</i>	<i>Bitis ari- etans</i>	<i>Bitis gabor- ica</i>	<i>Naja haje (S.A.)</i>	<i>Naja nigricollis</i>	<i>Sepedon haemachates</i>	<i>Dendraspis angusticeps</i>	<i>Naja melanoleuca</i>	<i>Bitis nasuta cornuta</i>
Serum I	3.0	25.0	—	3.3	3.6	3.0	1.2	1.1	7.0
Serum II	1.9	24.0	20.0	2.4	2.4	1.7	0.8	0.5	6.0
M l d	0.35	1.5	2.0	0.9	1.2	1.0	0.35	0.35	1.8

It appears from these results that the titres of these two sera against the venoms of *N haje* (South African), *N nigricollis* and *S haemachates* come within close range of the specific *N flava* titre. However, the titres against the three first venoms amount to from two to three lethal doses only, whereas 1 c c antivenene neutralizes from five to nine lethal doses of *N flava* venom.

Regarding the venoms of *N melanoleuca* and *D angusticeps*, the amount of venom neutralized corresponds to from two to three lethal doses also, although the titres appear considerably lower when taken as the absolute weight

of venom. Considering more especially the venom of *D. angusticeps* the difficulties in collecting sufficient quantities of this relatively uncommon colubrine make it gratifying to see that some increase in potency results from enzyme-purification. The same applies to the venom of *V. melanocephala* of which so far limited supplies were obtained through the kindness of Dr CECCALDI Director of the Institut Pasteur Brazzaville.

The antivenene seems to give some protection against the equatorial viper *Bitis nasicornis*.

#### AVIDITY OF ENZYME PURIFIED ANTIVENENE.

Avidity is here taken to mean the speed of union between antibody and venom a matter of paramount importance when one considers the rapid action of venom introduced in the system.

A set of mixtures was made of a constant volume of antivenene (S. 5883) and increasing doses of *A. ferox* venom. The volume in each tube was adjusted with saline, so that a mouse received 0.2 c.c. of serum in a volume of 0.5 c.c. 0.5 c.c. of this mixture was injected intravenously into mice of 20 grammes immediately the mixtures were made eight mice being used for each mixture. This was repeated after half an hour and after 1 hour at 37° C. Another antivenene (S. 5949) was titrated against *B. arsiensis* venom. Each mouse received 0.1 c.c. of serum in a total volume of 0.5 c.c. Otherwise the technique was as described above. The results are recorded in Table III.

TABLE III.

TITRATION OF ANTIVENENE AGAINST VENOM OF *B. arsiensis* AND *A. ferox* IN MICE, TO SHOW THE SPEED OF UNION BETWEEN ANTIBODY AND VENOM. THE MIXTURES WERE INJECTED AFTER DIFFERENT PERIODS OF CONTACT. 37°

	S 5883— <i>A. ferox</i>				S 5949— <i>B. arsiensis</i>			
Mg. cecum per dose	0.22	0.40	0.18	0.8	0.7	0.4	0.3	
C.c. of antivenene per dose	0.20	0.40	0.40	1	0.1	0.1	0.1	
Rate of survival after contact at 37° C								
for 0 hours	0.8		8.8	2.8	1/8	7/8	8/8	
1/2 hour	0.8	7.8	8.8	1.8	4/8	7/8	8/8	
1	0.8	8	8.8	2.8	2.8	8.8	8/8	

These results seem to indicate a high speed of union between the venom and its antibody.

#### CONCLUSION

Enzyme-purification of polyvalent South African *Naja ferox* *Bitis arsiensis* antivenene and polyvalent *Bitis gabonica* antivenene resulted in from four to

six times concentration, and an increase in nitrogen content to approximately double the original amount. Group neutralizing antibodies against other African colubrine venoms, namely, *N melanoleuca*, *N haje*, *N nigricollis*, *Sepedon haemachates*, and *Dendraspis angusticeps*, were concentrated to useful therapeutic levels. Limited protection was observed against the venom of *Bitis nasicornus*. The speed of union between enzyme-treated antivenene and venom is high.

The increased antibody concentration and purity of enzyme-treated antivenene marks a big therapeutic advance in the treatment of snake bite, as compared with the antivenene previously prepared by fractional sodium sulphate precipitation, and its use reduces the risk of serum reactions.

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## ZINC SULPHATE FLOTATION OF FAECES

BY

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The zinc sulphate flotation technique for the concentration of ova and cysts in faeces elaborated by FAUST and his collaborators (1938)\* is, despite its complexity, gaining popularity with all who have tried it.

The technique consists in repeatedly washing by centrifugalization a sieved specimen about the size of a walnut. When the supernatant fluid is clear, the sediment is re-suspended in a solution of zinc sulphate of an s g of 1.180, re-centrifuged and the top layers transferred to a slide, mixed with iodine, covered and examined. Commercial zinc sulphate is a somewhat variable quantity, so it is as well to determine the s g by means of a hydrometer (Bé 22.12) or by other means. If anhydrous  $\text{ZnSO}_4$  is used 186.6 grammes per litre should give the desired density, as should 333 grammes per litre of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ . Generally speaking, specimens containing much mucus are not suitable for this technique, as the mucus floats *in toto*, and there is little gain in concentration.

\* FAUST, E C , D'ANTONI, J S , ODOM, V , MILLER, M J , PERES, C , SAWITZ W , THOMEN, L F , TOBIE, J , and WALKER, J H (1938) *Amer J trop Med*  
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TABLE II.

RELATIVE EFFICIENCIES OF DIRECT EXAMINATION AND ZINC SULPHATE FLOTATION.

Parasite	Direct + %	Zinc + %	Both + %	Zinc Ratio $\frac{\text{Zinc}}{\text{Direct}}$
<i>E. histolytica</i>	49.5	96.6	25.7	173.8
<i>E. coli</i>	51.3	92.7	47.0	179.8
<i>E. magna</i>	28.9	91.2	29.2	318.2
<i>I. butschlii</i>	16.4	91.5	10.9	877.8
<i>G. lamblia</i>	18.8	92.9	12.5	360.9
<i>C. normalis</i>	23.3	100.0	22.3	269.0
<i>A. lumbricoides</i>	76.6	91.1	76.6	125.6
<i>T. trichiura</i>	46.8	95.8	41.4	401.9
<i>O. crassus</i>	—	100.0	—	—
<i>Antylocheilus</i> spp.	17.7	93.3	13.0	836.2
<i>Taenia</i> spp.	68.2	89.0	35	95.2
<i>S. mansoni</i>	83.6	81.3	19	85.7
All positives	51.6	92.5	44.1	179.2

It is apparent that in this technique we have a very valuable aid to diagnosis of intestinal parasites. However the direct film remains as the method of choice, as it reveals the trophozoites in addition. Laboratories with adequate facilities should perform both techniques.

## SUMMARY

The direct faecal film and FAUER's zinc sulphate flotation technique are compared in a series of 1,539 specimens. The overall gain by the use of this technique is 179 per cent.

# THE SEDIMENTATION RATE IN THE AFRICAN PEASANT WITH SPECIAL REFERENCE TO TRYPANOSOMIASIS

BY

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There are few records of sedimentation rate (S.R.) findings in normal Africans or in those suffering from trypanosomiasis. LASSMANN (1939) gives the following figures (Westergren method) for mines labourers in the Belgian Congo (All normal Europeans less than 20 mm in 1 hour)

	0-10	10-20	20-30	30-40	40-50	50-60	60 mm +
Healthy African males, 1,258	65	264	541	245	103	14	26
Tuberculous African males, 89	—	—	—	9	20	45	15
African males with tropical ulcer 191	25	48	35	9	21	22	31

No explanation for the high sedimentation rates of those apparently normal was found.

The closely related phenomenon of autoagglutination was investigated by TODD (1910). He found autoagglutination in 395 out of 1,406 Congo natives. Trypanosomes were found in 183 of those with agglutination and in twenty-four of those without. Autoagglutination was therefore present in 212 out of 1,999 (17.8 per cent) persons in whom no cause for it could be found. DUBOIS (1912) concluded that the prevalence of autoagglutination was due to the multiplicity of tropical parasites and that its absence was strong evidence against trypanosomiasis. In the 1930 *Annual Report of the Nigerian Sleeping Sickness Service*, the S.R. is said to be increased in human trypanosomiasis and the increase is said to correspond roughly with the severity of the disease. Increased S.Rs

\* Our thanks are due to the DIRECTOR OF MEDICAL SERVICES, Nigeria, for permission to publish, to many past and present members of the SLEEPING SICKNESS SERVICE for criticism and advice, and to Dr A. J. DUGGAN for help in following up some cases.



in various animals infected with a variety of trypanosomes have been reported by FRENCH (1937) and NICOLLE and SIMONS (1939). LAWSON (1942) reported high S.R.s in Uganda natives with trypanosomiasis and found it of no value as a test of cure.

This paper is an attempt to assess the value of the sedimentation rate as an aid in the management of human trypanosomiasis under field conditions of mass survey and treatment having regard to the high rates frequently found in apparently normal Africans. Sedimentation rates have been done with a tube made by Mesara. Hawkley shown in Fig. 1



FIG. 1

Five per cent. sodium citrate is run up to the mark 'C'. Capillary blood from the finger is run into the tube behind the citrate until the mixture reaches the mark 'B'. The mixture is then run out on to a clean microscope slide and thoroughly mixed, the mixture is then run up to the mark 'O', an elastic band is stretched round the ends of the tube which is then placed in a rack. Readings have been taken at 5 to 85 F. The proportion of blood to citrate is four to one. *Using so short a tube it is found that when the S.R. is extremely rapid as in cases of trypanosomiasis the 10-minute reading is largely a measure of the intensity of agglutination and the 1-hour reading is an approximation to the packed cell volume. The tube can be centrifuged and packed cell volume reading obtained. The speed with which large numbers of estimations can be made without syringes and sterile precautions make the method ideal for ruminant tropical practice.*

## FACTORS AFFECTING THE SEDIMENTATION RATE IN TRYPANOSOMIASIS

Three factors known to affect the sedimentation rate are commonly present in human trypanosomiasis (1) Anaemia. (2) Plasma protein changes. (3) Autoagglutination.

### (1) ANAEMIA.

The average packed cell volume of sixty-six males and twenty two females with untreated trypanosomiasis was 30 per cent. and 28 per cent. respectively. This degree of anaemia is not sufficient alone to account for the very high sedimentation rates found in this disease.

### (2) PLASMA PROTEIN CHANGES.

SECK and BOXER (1936) give the normal serum total protein as 7.8 to 8.5 grammes per litre in all cases of trypanosomiasis with spinal fluid changes

they find that the total protein exceeds 100 grammes per litre. The increase is mainly due to increased globulin, the albumin being sometimes more and sometimes less than normal, the albumin-globulin ratio is reversed. It has not been possible to do plasma protein estimations under "bush" conditions, but in kala-azar, a protozoal disease producing similar plasma protein changes, SHELLIM (1944) gives the following figures for a Greek soldier suffering from kala-azar.

	Total protein grammes %	Albumin grammes %	Globulin grammes %	Albumin globulin	S R mm in 1 hr
Normal	7.2	4.5	2.7	1.7 1	2-10
Before treatment	10.8	2.8	7.9	0.35 1	150
Between courses	13.0	1.3	11.7	0.11 1	140
After treatment	10.6	3.30	7.21	0.47 1	100
4 months later	9.7	5.5	3.22	1.4 1	20

It was not stated whether autoagglutination was present or not. In two cases of trypanosomiasis in which the S R was more than 10 mm in 10 minutes the plasma protein albumin globulin ratio was 0.3 1.

### (3) AUTOAGGLUTINATION

Lack of space prohibits discussion of the precise relationship of autoagglutination to the presence of cold agglutination and the relationship of both to the sedimentation rate. Suffice it to say that the writer believes that cold agglutination is a factor of no importance affecting the S R at tropical temperatures, although its presence can always be demonstrated.

The following records of sedimentation rates in Africans have been made in Benue Province, Nigeria, an area about the size of Scotland, containing 1,200,000 inhabitants. The Sleeping Sickness Service started mass survey in 1937 and in the next 5 years examined about 1,000,000 people and found and treated about 70,000 cases of trypanosomiasis, the infection rate varied from 50 per cent in some areas to less than 1 per cent in others. Thirty treatment centres have been established and these continue to treat about 4,000 new and relapsed cases annually. R-surveys in the areas previously most heavily infected now show infection rates of 0 to 2 per cent. The writer has been fortunate in being associated with this work from 1938 to the time of writing. Sedimentation rates have been recorded in a large number of cases. The 10 minute reading has been used at shade temperature (75° to 85° F). Since the 10-minute reading in Europeans is not readily measurable, being a fraction of a millimetre, Fig. 2 shows the percentage distribution of the 1-hour S R

reading of twenty-seven European males in Benue Province, 301 healthy African males, and 287 African males with trypanosomiasis.

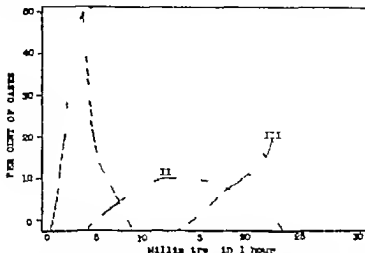


FIG. 2.—Per cent. frequency distribution of sedimentation rates of I, twenty-seven European males in Benue Province II 301 healthy African males III 287 African males with trypanosomiasis.

The normal European sedimentation rate is less than 5 mm. in 1 hour. The majority of Africans have 1 hour readings between 10 mm. and 15 mm. Most cases of trypanosomiasis have rates above 20 mm. in the hour and, as already mentioned, this is an approximation to the packed cell volume.

Table I shows the sedimentation rate in millimetres in 10 minutes of 591 Africans suffering from trypanosomiasis and of 654 normals.

For each sex two main divisions have been made in each group. Those with trypanosomiasis are divided into those with new infections and those with re-infections. The normals are divided into those never treated for trypanosomiasis and those treated more than 3 years previously but symptom-free and believed cured at the time of examination.

Various tribes are represented in the analysis and each has been treated as a sub-group of the main division. The ages of patients are not accurately known: the majority are young or middle-aged adults: the group of schoolboys does not differ significantly from those of adults in the same area and it is believed that age is not a cause of variation of S.R. except perhaps at the extremes of life. Readings have been taken during all months of the year from 1942 to 1943: no single group has been examined regularly in every month to see if there is any seasonal variation, but since groups with both very high and very low average S.R. have been found in the same month in different areas it is probable that place is a more important cause of variation than season. Whether the difference between groups is due to difference of race or of place has not yet been determined.

Despite the fact that the groups among normals are not individually comparable with the groups among the infected, the two groups as a whole may reasonably be compared since both are drawn from the population in all corners of Benue Province.

Among the normals, male S R s do not differ significantly from those of females but data are not really adequate to settle this point (Table I, pp 222 *et seq*)

There is no difference between those with and without filariasis, or between those with and without schistosomiasis, or between those with a history of past yaws and those without. More data might reveal significant differences but it is clear that they would not be large. Among those with trypanosomiasis seen in hospital there was no significant difference between those with symptoms and those without. Among 140 cases seen at Makurdi Hospital, in which a purely clinical assessment was made without knowledge of the spinal fluid changes, it was found that —

In 74 per cent of cases there was close agreement between S R and clinical state

In 16 per cent of cases there was definite disagreement between S R and clinical state

In 10 per cent of cases the clinical state was difficult to assess. Comparison of S R. and cerebrospinal fluid changes was made in 252 cases of trypanosomiasis seen in dispensaries and on re-surveys in the year 1943. The cases are fairly typical of those found since mass survey has been completed. They have been divided into five groups

Group 1 Trypanosomes in gland juice CSF normal  
Group 2 Trypanosomes in gland juice CSF Cells + Protein normal

Group 3 Trypanosomes in gland juice CSF Cells + Protein +

Group 4. No trypanosomes demonstrable in blood or gland juice CSF abnormal

Group 5 Nervous relapse after treatment Trypanosomes rarely demonstrable CSF abnormal

	Cases	Mean S R mm in 10 minutes	Mean C S F	
			Cells per c.mm	Protein mg %
<b>Males</b>				
Group 1	35	9.71 ± 1.03	2	15
2	47	13.43 ± 0.83	27	17
3	36	16.83 ± 0.84	233	43
4	15	10.5 ± 1.6	247	39
5	44	3.88 ± 0.48	151	46
<b>Females</b>				
Group 1	12	10.10 ± 1.49	2	15
2	21	14.52 ± 1.30	42	18
3	16	17.97 ± 0.83	271	38
4	11	11.27 ± 1.94	127	32
5	15	2.73 ± 0.54	109	45

MALES NEVER TREATED FOR TRYPAONOSOMIASIS.

Group.	Number of cases.	Mean $\pm$ S. R. a. mm in 10 minutes	Frequency distribution per cent.			
			0-4 mm.	5-9 mm.	10-14 mm.	15+ mm.
African recruits, healthy ... ..	42	3.05 $\pm$ 0.45	78.6	11.9	2.5	—
...	39	1.17 $\pm$ 3.11	100.0	—	—	—
...	36	2.67 $\pm$ 0.45	83.3	3.3	2.6	—
Tiv men as labourers, had years ... ..	29	17 $\pm$ 0.43	63.1	17.9	—	—
...	23	2.0 $\pm$ 0.28	93.3	7.1	—	—
... never had years	23	1.43 $\pm$ 0.19	100.0	—	—	—
Tiv school boys, healthy	39	2.13 $\pm$ 0.13	84.6	3.1	—	—
... all with schistosomiasis	12	2.04 $\pm$ 0.28	100.0	—	—	—
Bassaka peasants	31	4.07 $\pm$ 0.61	84.5	23.6	3.7	—
Gatari ... ..	6	6.83 $\pm$ 0.83	18.7	68.7	16.7	—
Gargel ... ..	9	4.91 $\pm$ 1.93	77.8	11.1	—	11.1
Bantah ... ..	13	1.6 $\pm$ 0.34	94.6	5.6	—	—
Tiv Nysev ... ..	14	3.36 $\pm$ 0.79	71.4	21.4	7.1	—
Penda ... ..	17	3.80 $\pm$ 0.48	84.7	35.3	—	—
Toto ... ..	25	3.3 $\pm$ 0.53	78.0	20.0	4.0	—
Males with filariasis ... ..	13	1.2 $\pm$ 0.41	93.3	7.7	—	—
Total	319	3.9 $\pm$ 0.13	85.1	11.9	2.3	0.3
MALES TREATED FOR TRYPAONOSOMIASIS 3 OR MORE YEARS PREVIOUSLY. —LOW SYMPTOMATICS						
Bassaka ... ..	44	4.6 $\pm$ 0.80	84.6	29.3	16.0	—
Donga ... ..	22	4.23 $\pm$ 0.84	73.7	13.6	9.1	4.5
Gatari ... ..	9	3.46 $\pm$ 0.87	86.7	33.3	—	—
Gargel ... ..	8	8.3 $\pm$ 0.23	80.0	70.0	—	70.0
Bantah ... ..	13	1.38 $\pm$ 0.35	100.0	—	—	—
Tutu ... ..	29	3.03 $\pm$ 0.78	73.8	17.2	3.4	3.4
Turka Tiv ... ..	30	3.7 $\pm$ 0.80	78.7	13.3	6.7	3.3
Tiv Nysev ... ..	11	4.03 $\pm$ 6.08	90.9	—	9.1	—
Gibeta ... ..	13	1.40 $\pm$ 0.25	100.0	—	—	—
Toto ... ..	27	3.44 $\pm$ 0.41	81.5	14.8	3.7	—
Total	203	3.47 $\pm$ 0.28	74.9	16.3	6.9	2.4
All male normals treated and never treated	662	2.9 $\pm$ 0.13	81.9	13.3	4.2	3.3

## MALES WITH TRYPA NOSOMIAS NEW CASES

Group	Number of cases	Mean $\pm$ S.E. mm in 10 minutes	Frequency distribution per cent			
			0-4 mm	5-9 mm	10-14 mm	15+ mm
1941 Makurdi with symptoms	68	14.7 $\pm$ 0.59	2.9	11.8	27.9	57.4
" " no "	19	11.79 $\pm$ 1.1	10.5	21.1	26.3	42.1
1942 F. Tiv survey	100	12.92 $\pm$ 0.73	7.1	20.8	31.1	40.6
1943 Wulari spo. survey	17	13.35 $\pm$ 1.47	11.8	11.8	23.5	52.9
1943 Turu Tiv	4	12.63 $\pm$ 2.43		25.0	25.0	50.0
1943 Turu Tiv	13	11.5 $\pm$ 1.31		15.4	23.1	61.5
1943 Guita	21	12.14 $\pm$ 1.78	9.5	33.3	9.5	47.6
1943 Panda	15	10.72 $\pm$ 1.61	33.3	11.8	11.8	41.2
1943 Njivi Tiv	7	10.56 $\pm$ 1.97	14.3	28.6	14.3	42.9
Miscellaneous C.S.F. normal	35	9.7 $\pm$ 1.03	22.9	31.4	22.9	22.9
C.S.F. cells + and protein normal	47	13.43 $\pm$ 0.83	6.4	23.1	23.4	46.8
" + " +	36	10.83 $\pm$ 0.61		10.6	10.7	77.8
Total new cases	390	13.19 $\pm$ 0.29	8.7	19.0	24.1	47.9

MALES INFECTED WITH TRYPA NOSOMIAS						
1942 Turu Tiv	8	14.75 $\pm$ 2.02		25.0	12.5	62.5
1943 Guita	15	9.3 $\pm$ 1.71	40.0	13.3	13.3	33.3
1943 Panda	23	8.78 $\pm$ 1.12	26.1	10.4	26.1	17.4
	46	9.99 $\pm$ 0.92	26.1	21.9	19.6	30.4
Total males with trypanosomiasis	436	12.85 $\pm$ 0.28	10.6	19.5	23.9	16.1

TABLE I—

FEMALE NORMALS  
NEVER TREATED FOR TRYPAKOSOMIASIS.

Group	Number of cases.	Mean $\pm$ S. R. c.	Frequency distribution per cent.			
			0—4 mm.	5—9 mm.	10—14 mm.	15+ mm.
Various	20	3.03 $\pm$ 0.80	75.7	20.0	3.3	—
With filariasis	17	4.91 $\pm$ 0.73	64.7	29.4	5.9	—
Total females never treated	4	3.90 $\pm$ 0.45	7.3	23.4	4.3	—
FEMALES TREATED FOR TRYPAKOSOMIASIS 3 YEARS PREVIOUSLY BELIEVED CURED.						
Jato Aka	11	4.09 $\pm$ 0.83	84.8	47.3	—	—
Nyiriv Tiv	4	1.73 $\pm$ 0.41	100.0	—	—	—
Total treated	15	3.47 $\pm$ 0.84	66.7	23.3	—	—
All females normal	62	3.11 $\pm$ 0.34	71.0	25.0	3.3	—
Pregnant females	34	0.04 $\pm$ 1.70	27.3	16.7	23.9	39

The statistical analysis (p. 221) of Table I and subsidiary tables (pp. 223 *et seq.*) which is discussed in detail in the work of Dr. E. L. Lumsden of the Medical Research Council's statistical staff.

continued

TABLE WITH TRYPAISO OMIACT  
NEW CASES

Group	Number of cases	Mean S.R.	S.E.	Frequency distribution per cent			
				0-1 min	1-2 min	10-14 min	15+ min
Makurdi + 1 sample	28	16.2 ± 0.64			7.6	5.0	71.4
E. Try survey	8	17.81 ± 1.19			73.7	25.0	7.0
Wulari per survey	6	16.83 ± 1.83		100		50.0	10.7
Litur	2	14.5 ± 1.77			22.2	11.1	11.7
Turan Tiv	9	14.67 ± 1.46		22.2	11.1	44.4	22.2
Gitata	9	9.79 ± 1.7		5.5	5.5	42.1	47.4
Panda	19	17.89 ± 0.93				11.7	8.8
Nyiet Tiv	7	20.86 ± 1.3					
Miscellaneous—							
(1) Normal CSI	12	10.69 ± 1.49		16.7	25.0	73.7	25.0
(2) CSF cells + normal protein	21	14.62 ± 1.59		18	23.8	19.0	52.4
(3) protein +	16	17.95 ± 0.83				18.8	81.2
Total female new cases	137	14.82 ± 0.45		5.1	10.9	27.0	77.0

REINFECTED FEMALE CASES							
Turan Tiv	6	10.92 ± 1.41			50.0	33.3	16.7
Gitata	4	12.75 ± 2.92				50.0	50.0
Panda	8	12.91 ± 2.12		25.0	12.5		62.5
Total females reinfected	18	12.22 ± 1.26		11.1	33.3	11.1	44.4
All females with trypanosomiasis	165	14.52 ± 0.43		5.8	11.5	25.2	55.5

\* S.R. mm in 10 minutes



TABLE 1—*continued*

Group.	Number of cases.	Sedimentation rate mm. in 10 minutes.				
		Mean $\pm$ s.	Per cent. frequency distribution			
			0—	5—	10—	15+ mm
All trypanosomiasis normals	581	12.29 $\pm$ 0.34	0.3	17.9	24.1	45.6
	834	2.95 $\pm$ 0.12	80.8	14.5	4.1	0.8
Difference		10.37 $\pm$ 0.55				

*The difference is nearly 20 times the standard error (s.e.), highly significant one*

Comparison of all males with trypanosomiasis, with all infected females shows significant difference.

Group.	Number of cases.	Sedimentation rate mm. in 10 minutes.				
		Mean $\pm$ s.	Per cent. frequency distribution			
			5—	8—	10—	15+ mm
Males (trypanosomiasis)	436	12.85 $\pm$ 0.28	10.4	19.5	23.9	44.1
Females	185	16.53 $\pm$ 0.43	8.8	13.8	25.2	53.3
Difference	---	1.47 $\pm$ 0.51				

Comparison of all new cases of trypanosomiasis with all reinfected cases shows a significant difference, the reinfected having lower S.R. than the new. This comparison is not, however, fair one as all the reinfected cases came from three centres—Turin, Ghata and Panda. Comparing the two groups from these centres only the same difference is apparent but is not statistically significant.

Group.	Number of cases	Sedimentation rate mm. in 10 minutes				
		Mean S.R. $\pm$ s.	Per cent. frequency distribution.			
			0—	5—	10—	15+ mm
Turin, Ghata, Panda						
New cases	83	12.49 $\pm$ 0.6	12.3	17	22.7	41.7
Reinfected cases	64	10.45 $\pm$ 0.78	21.9	26.8	17.2	34.1
Difference		1.83 $\pm$ 0.97				

TABLE I—*continued*

Among the normals, cases previously treated are not comparable as a group with those never treated. The only places where these groups are both represented are Bissualla, Gatari, Gargei, Bantagi, Nviei and Toto. When these are compared no significant difference is apparent.

Among the normals, cases previously  
 those never treated The only places where these  
 Bissualla, Gatari, Gargei, Bantagi, Nviei and Toto When the  
 significant difference is apparent

Group	Number of cases	Mean $\pm$ s.e.	Sedimentation rate mm. in 10 minutes			
			Per cent frequency distribution			
			0—	5—	10—	15—mm
Bissualla, Gatari, Gargei,	103 109	3.7 $\pm$ 0.34	71.8	21.3	5.8	1.0
Bantagi Nviei, Toto		3.6 $\pm$ 0.30	71.5	19.3	8.3	0.9
Never treated						
Treated 3 years		0.1 $\pm$ 0.45				
Difference						

arranged according to distribution of S R level

The first three groups, arranged according to distribution of S R levels, were —

Males S R	0—9 mm	10—14 mm	> 15 mm	Females S R	0—9 mm	10—14 mm	> 15 mm
Group 1	54%	23%	23%	Group 1	42%	33%	25%
2	30%	23%	47%	2	28.5%	19%	52.5%
3	5.5%	10.5%	75%	3	0%	10%	81%

These three groups show clearly that where trypanosomes are present in gland juice the S R is correlated with C S F changes. Groups 4 and 5 show that there is no direct causal relationship and suggest that a high S R is due to the presence of trypanosomes in the circulation.

There are two ways in which the association of high S R and C S F changes might be explained, it may be that the S R rises as the disease progresses so that by the time the nervous system is affected it is high, or it may be that in a case with a high S R the nervous system is more likely to become involved.

However, since writing this, work in another area where the "normal" S R was extremely high has shown that this correlation is not always present. From an area outside Benue Province, Dr A J DUGGAN has found the following —

Fifteen cases (eight males, seven females) with normal C S F, 16.4 mm in 10 minutes

Seventeen cases (ten males, seven females) with abnormal C.S.F., 16.4 mm. in 10 minutes.

Fifty-six normals in the same area had the very high average S.R. of 6.3 mm. in 10 minutes.

Yaws, leprosy, gonorrhea, filariasis and helminthiasis were rampant in the area.

Cases of trypanosomiasis observed over a period of 70 days without treatment, the S.R. showed no tendency to rise or fall.

### Example

Twenty Tiv tribe (males and females). Average S.R. at diagnosis, 16.2 mm. in 10 minutes.

Same cases, 70 days later without treatment, 16.2 mm. in 10 minutes.

Observation of a group of twenty-three exceptionally mild cases over a period of 20 months showed that trypanosomes tended to disappear from those with low and to persist in those with high, S.R.

During the 1st year there was practically no change in the average S.R. though considerable individual variations occurred. In the last 8 months, however a significant rise of S.R. occurred in the group in which trypanosomes persisted, and this coincided with a deterioration in their clinical condition. The two groups are shown in Table II (a) and (b).

The difference between Groups (a) and (b) in each of the three observations is statistically significant so is the difference between Group (a) in April, 1944 and in December 1944. In Group (b) no trypanosomes were found in fifty-four examinations. In Group (a) trypanosomes were found on forty-five occasions out of sixty-three blood or gland juice examinations.

The S.R. changes in one case which had to be treated after 6 months' observation form an interesting contrast.

### Example

MALAMBELE. MALE, AGE 40 WITH TRYPANOSOMES. GLAND JUICE.

	S.R. mm. in 10 minutes.	Cells.	C.S.F. Protein.
At diagnosis (March, 1943) ...	18	—	—
Oct., 1943 (7 months without treatment. Trypanosomes still in gland juice)	15	44	21 mg.
April, 1944, after treatment with entypol 1 gramme × 3; trypanamide 2 gramme × 10 every 2nd day	1	8	18



TABLE III.

TWENTY CASES OF TRYPANOSOMIASIS FROM A MILD DISTRICT. DIAGNOSED BY GLASS PUNCTURE AND GIVEN SINGLE INJECTION OF ANTEYFOL. (1 GRAMME FOR ADULTS)

		Sedimentation rates in mm. in 10 min.			C.S.F.	
		Mar 1943	Apr 1944	Dec., 1944	Oct 1943.	Apr 1944.
		At diagnosis.			Cell. Protein.	Cell. Protein.
*Tara ...	ML	18	2½	1	2 — 15	0 — 1.
*Sadin	ML	18	2	11	8 — 18	2 — 8
Baw	ML	15	8	12	8 — 18	1 — 15
Datoro	ML	21	4½	1½	0 — 18	C.S.M
Ude ...	ML	20	3	12	25 — 38	100 — 70
Ado	ML	1	10	7	2 — 18	8 — 20
Ashi	ML	8	8½	8	8 — 12	4 — 1
*Copo	ML	4½	2	8	8 — 18	15 — 18
T	ML	8	3	4	0 — 18	—
Oja ...	ML	8	½	1	2 — 15	0 — 12
*Ure	ML	8	1	3	8 — 21	0 — 24
Kabo	ML	17	3	10	11 — 18	8 — 18
Mam	ML	18½	7	12	1 — 15	11 — 15
Ag	ML	7	2½	12	0 — 18	—
*Tani	F	20	8	10	185 — 30	74 — 42
Nadji...	F	18	7½	7	18 — 21	1 — 12
Nim	F	8	8½	4	23 — 20	0 — 12
Joro	F	7	1	4	8 — 19	1 — 14
Ladi ...	F	19	4	10	1 — 12	1 — 1
Faru	F	17	1	8½	—	0 — 12
Average of 20 cases		12.9	4	6.5		

Cases marked with \* had been treated in 1937 and are regarded as re-infections.

injection. Some cases were almost certainly cured. It is interesting to note that the two cases with considerable C.S.F. changes had S.R. above the average at diagnosis and at the end of observation. All cases were subsequently treated. Tani had been sent for treatment in April, 1944 but refused to attend the dispensary. Datoro was suffering from cerebrospinal meningitis in April, 1944 and had a S.R. of 15 mm. in 10 minutes. He is shown with a S.R. of 4½ mm. in 10 minutes as this was his S.R. in October 1943. He recovered after treatment with sulphapyridine.

Variation in resistance is probably a more potent factor in causing variation in S.R. than in variation of virulence. This is illustrated in figures obtained at Banki, a mining district in Zaria Province. In an area of about 5 square miles there is a population of about 700 mines labourers and 900 local

population The labourers and peasants mix freely and it is hard to believe that the former could be infected with a virulent, and the latter with a mild, strain of trypanosome Both groups are examined regularly and it is practically certain that no case in either group had been infected more than 6 months at the time of examination Nevertheless, the S R s show a considerable difference

*Example*

	S R mm in 10 minutes
Average S R. of 30 mines labourers (males) with trypanosomiasis	14.26 $\pm$ 0.67
Average S R of 25 peasants with trypanosomiasis (18 males average S R 9.6 $\pm$ 0.94, 7 females average S R 10.3 $\pm$ 6.55)	9.4 $\pm$ 0.81
Difference =	4.86 $\pm$ 1.05*

\*This is about four times the standard error which is significant Had the peasant group consisted only of males the difference would probably have been greater (This Table not analysed by E L-F)

While most of the labourers admitted symptoms, i.e., unable to work hard, none of the local population did so

In both groups trypanosomes were easily found in a wet blood film in about 30 per cent of cases—a sign of early infection and probably accountable for the great transmissibility of the infection There is no difference in S R between cases in which blood trypanosomes are easily found and cases in which they are not Most observers agree that the infection in mines labourers is severe, and this is usually attributed to exalted virulence due to rapid passage from man to man Lowered resistance seems more probable in this instance This might be due to the greater incidence of other diseases, poor living conditions or, most probably, harder work In cattle, exercise is a sure method of activating latent trypanosomiasis That fatigue does raise the S R is shown in the following example a Tiv male, treated for trypanosomiasis with 10 grammes antypol and believed cured at the time of examination, though subsequently he suffered a nervous relapse

In the treatment of trypanosomiasis interest is usually centred on the changes in the nervous system To the epidemiologist, this is of secondary importance It cannot be over-emphasized that as a cause of depopulation in Nigeria the parasitaemia is of primary importance Even in cases with considerable C S F changes it is usually the parasitaemia rather than the encephalitis which kills The relatively uncommon case in which the blood

*Example*

S.R. before 20 miles trek. 29.3 42.				S.R. after 70 miles trek.	
10 minutes. 1 hour					
	2	—	16	4½	— 17
	1	—	16	4	— 18
	1½	—	16	7½	— 17
	2½	—	17	6	— 18
	2½	—	17	6	— 18½
Average of four readings	2	—	16	Average of five readings	5.4 — 17.4

infection seems to have dried out while the brain is still infected comes to one's notice eventually as the village-idiot type—such cases die from inability to look after themselves rather than as the direct result of the disease.

The S.R. is the best guide to the severity of the blood infection. Symptoms are a good guide only when one is intimately acquainted with the patient. On mass survey this is impossible. There is no evidence that cases with high S.R. are more difficult to cure except in that they are often associated with advanced nervous involvement.

In testing the trypanocidal activity of drugs it is important to remember that in cases with low S.R.s trypanosomes frequently disappear for long periods without any treatment at all and some cases may even be spontaneously cured.

Perhaps the most valuable use of the S.R. is in the diagnosis of cryptic cases. On mass survey S.R.s should be done on all cases in which trypanosomes cannot be found. Those with high S.R. are given a single injection of antypol or pentamidine and re-examined when treatment starts a month or more later. Those which show a pronounced fall of S.R. should be regarded as cases of trypanosomiasis.

The decrease in S.R. is positive evidence of the trypanocidal power of a drug and is valuable confirmation of the negative evidence of failing to find trypanosomes. It must, however, be stated that trypanosomes can be present in blood with a normal S.R. but this is rare. The idea that S.R. estimation can be a substitute for C.S.F. examination has been abandoned. To summarize

In trypanosomiasis (*T. gambiense*)—

(a) A low S.R. indicates an early infection of low virulence.

(b) A high S.R. may indicate an early infection of high virulence or a long standing infection of low virulence or concomitant disease. Whatever the cause of the high S.R. it probably carries a poor prognosis as it is an expression of the patient's lowered resistance.

# THE SEDIMENTATION RATE IN THE TREATMENT OF TRYPANOSOMIASIS

Groups of cases of trypanosomiasis treated by a variety of methods are shown in Table IV

Unfortunately, the groups are too heterogeneous and have been examined too irregularly for statistical comparison of their S R's. In general, the S R decreases daily by about 0.2 mm, but individual cases may respond much more rapidly. The daily decrease probably depends as much on the patient's physiological responses as on the trypanocidal action of the drug. The

TABLE IV

Group	Cases	Treatment*	Interval in days	Average S R. before	Average S R. after	Daily decreases
Wannune	20 M. & F	None	70	16.2	16.2	0.00
Tor Donga	29 M.	A, 0.2 x 1	74	13.4	6.9	0.09
Sankura	11 M.	A, 0.2 x 1	53	16.0	8.0	0.15
Gitata	12 M	A, 1.0	38	10.2	8.4	0.05
"	7 F	A, 1 x 1	38	11.7	6.4	0.14
"	11 M & F	A, 1 x 1	100	12.3	5.4	0.07
Makurdi	10 M & F	A, 0.2 x 1 A, 1 x 3	17	15.7	12.5	0.185
"	11 M & F.	A, 0.2 x 1 A, 1 x 3	22	15.5	9.3	0.28
"	5 M & F	(A, 0.2 T, 1) x 1 (A, 1 T, 2) x 3	19	17.6	12.2	0.32
"	19 M & F	(A, 0.2 T, 1) x 1 (A, 1 T, 2) x 3	23	15.4	9.4	0.26
"	7 M & F	T, 2 x 3	16	14.4	10.2	0.27
Wannune	7 M & F	(A, 1 T, 2) x 6	30	16.1	6.5	0.32
"	6 M & F	T, 2 x 6	30	16	9.1	0.23
"	3 M & F	A, 1 x 6	30	16	8	0.27
Birnawa	11 M & F	P, 0.1 x 10	10	20	16.5	0.16
"	11 M & F	P, 0.1 x 10	54	20	7.7	0.23
Zongon Aya	10 M & F	P, 0.1 x 5	36	15.4	5.05	0.28
Banki	9 M & F	(A, 0.5 T, 1.5) x 9	40	13.9	4.4	0.24
Makurdi	6 M	(A, 0.2 T, 1) x 1 (A, 1 T, 2) x 3 T, 2 x 5	50	16.3	3.9	0.25
"	6 M	A, 0.2 x 1 A, 1 x 3 T, 2 x 5	48	10.2	2	0.17
Jume	16 M	(A, 0.2 T, 1) x 1 A, 1 x 3 T, 2 x 5	45	12.2	3.8	0.19
Donga	11 M	A, 0.2 x 1 A, 1 x 3 T, 2 x 7	52	11.1	3.2	0.15
"	5 F	A, 0.2 x 1 A, 1 x 3 T, 2 x 7	52	15.2	5.3	0.19
Panda	11 M	A, 0.2 x 1 A, 1 x 3 T, 2 x 4	33	11.3	4.9	0.19
"	3 F	A, 0.2 x 1 A, 1 x 3 T, 2 x 4	33	17.2	11.2	0.15
Lafia	1 M	Anthiomaline, 4 c.c. x 8	55	14	2.5	0.21
"	2 M	N.A.B., 0.75 x	27	11.2	6	0.19

A = Antrypol T = Tryparsamide P = Pentamidine M = Males F = Females

\*First figure is number of grammes Second figure is number of injections



standard course of treatment for survey cases is eight injections, three of antypol, followed by five of trypanamide given every 5th day: this course takes 36 days. The average S.R. of survey cases is 13 mm. in 10 minutes: to fall to less than 5 mm. in 10 minutes, i.e. 9 mm. at 0.2 mm. per day would take 45 days. Severe cases which are likely to relapse are chiefly seen at dispensaries and are given thirteen injections every 5th day i.e. 65 days treatment. These cases average about 17 mm. in 10 minutes and therefore take about 63 days to return to normal. Although the S.R. is only a rough guide, it is reasonable when using this form of treatment to continue until the S.R. has returned to normal up to a maximum of twenty injections, i.e., 96 days.

It is not suggested that clinical progress should be ignored, but unfortunately the clinical aspect of the patient during treatment is an even poorer indication of his liability to relapse than at diagnosis. Nor is it suggested that C.S.F. findings should be ignored, but under present conditions—when about 18 000 cases are treated annually under the nominal supervision of three medical officers spread over an area the size of Britain—this method of controlling treatment is impracticable. A few cases have been seen in which the S.R. seems to have been a more reliable indication of failure than the C.S.F. finding and it is therefore worth doing in hospital practice where facilities are better for finding causes other than trypanosomiasis for a S.R. which does not return to normal.

Fig. 3 shows the effect of treatment on the S.R. of six groups of cases.

The C.S.F. of Group 1 cases might in some have returned to normal without further treatment and some of the cases in Groups 2 and 3 might eventually have relapsed, but there is no doubt that Group 1 as a whole has much the poorer prognosis.

In cases returning with nervous relapse after an adequate course of treatment, the S.R. is valueless.

Treatment of other diseases with high S.R. by antypol and trypanamide does not cause an appreciable fall in S.R.

#### THE SEDIMENTATION RATE IN VARIOUS DISEASES OF AFRICANS

To deal adequately with this subject a separate treatise would be required. A few somewhat dogmatic generalizations illustrating the value and limitations of the test in the mass diagnosis and treatment of trypanosomiasis are all that can be given here.

*Acute bacterial infections.*—In these the S.R. rises rapidly and soon returns to normal, e.g. pneumonia, meningitis. Since such patients are obviously sick, these conditions as a cause of high S.R. are not likely to be overlooked.

*Syphilis* is relatively uncommon in Benue though common in most of Northern Nigeria. The S.R. is high and intense autoagglutination is present

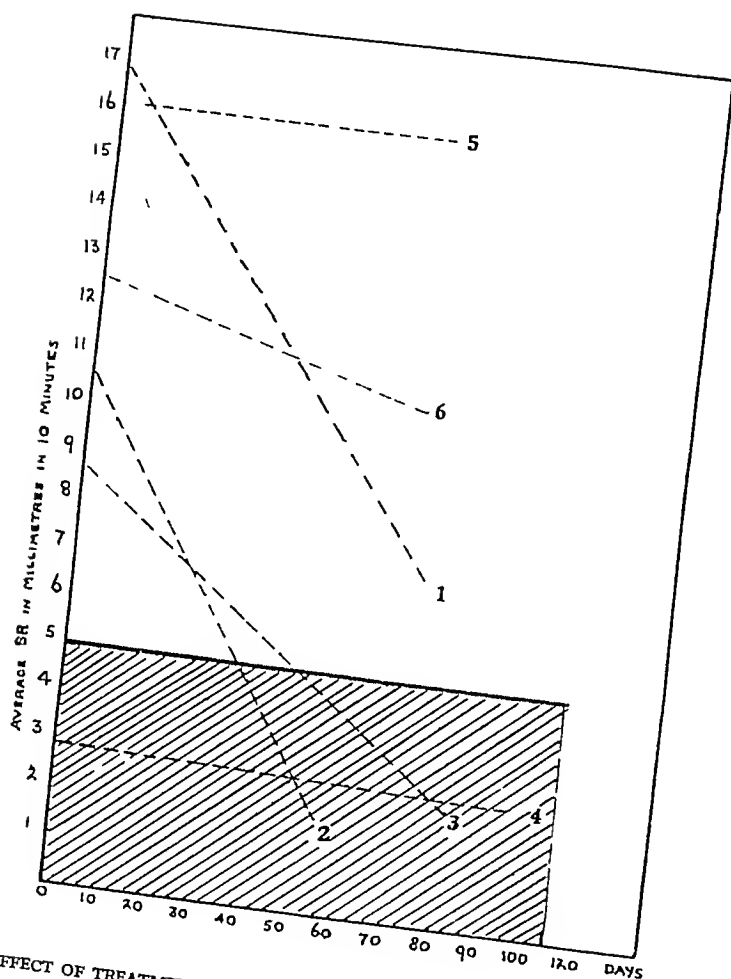


FIG 3—EFFECT OF TREATMENT ON THE SEDIMENTATION RATE IN TRYPANOSOMIASIS

- 1 Twenty cases with abnormal CSF after treatment
  - 2 Sixteen new cases with normal CSF after treatment
  - 3 Sixteen reinfected cases with normal CSF after treatment
  - 4 Sixteen cases with nervous relapse previously fully treated
  - 5 Twenty cases without treatment
  - 6 Five cases with raised sedimentation rates probably not due to trypanosomiasis—given trypanocidal treatment
- Shaded area shows limits of African "normal" in millimetres at 10 minutes

in all stages of the disease Since the spinal fluid cell count is often raised and enlarged neck glands are present, the differentiation from trypanosomiasis

may be impossible moreover the two may co-exist. However the high S.R. due to syphilis does not fall on treatment with antrypol as it does in trypanosomiasis. Treatment with N.A.B. however causes a fall of S.R. in both diseases.

Cases with high S.R. but no other signs, often give a history of syphilis.

*Yaws*.—Cases with the secondary eruption usually have a S.R. of 5 to 10 mm. in 10 minutes. Tertiary cases with ulceration have a very high S.R. Cases of clavus, juxta articular nodes and those with a history of yaws in childhood have normal S.R.

*Leprosy*.—The S.R. is usually abnormal. Sometimes very high but sometimes normal even in apparently active cases. Two cases of trypanosomiasis have been seen in which the S.R. did not return to normal with treatment. A year later both cases had leprosy which was not previously apparent. The proportion of grossly abnormal S.R.s is highest in districts where leprosy is rife. Since the incubation period may be 7 years, it is quite possible that many high S.R.s. are due to latent leprosy.

*Helminthiasis*.—*Ascaris Trichuris* and hookworm infestation do not appear to affect the S.R. except when severe anaemia is present.

*Filariasis*.—No increase in S.R. except in some cases of elephantiasis. Europeans with swarms of microfilariae in the blood have absolutely normal S.R.s.

*Schistosomiasis*.—S.R. is normal by African standards except when secondary infection or severe liver damage is present.

*Chronic nephritis and nephrosis*.—S.R. usually high.

*Tropical ulcer*.—S.R. very variable. Cases with rapidly extending ulcers have high S.R.s which fall on treatment.

*Gout*.—There is a marked association of gout with leprosy. Cases with gout do not differ from those without in the same area.

*Trypanosomiasis*.—That this may be a cause of high S.R. without any other sign is apparent from the rest of the paper. It should be remembered that in women pregnancy is a cause of higher S.R.

*Tuberculosis*.—Very high S.R.s are usually found.

The meaning of the high S.R. of the apparently normal African cannot be fully discussed here. It is almost certainly not racial. Dr J. C. BROWN has kindly consulted leading American haematologists, who report no difference in S.R. of normal American negroes and whites. Recent unpublished work by the Army Research Unit at Accra has found that the majority of normal African troops have a plasma protein albumin-globulin ratio of about unity as compared with 1.7:1 in the European. This is almost certainly the basis of the difference in S.R.s. The writer was unable to cause any decrease in S.R. in a group of thirty schoolboys by treatment with mepracrine for 12 days. The

S R of a group of well-fed soldiers was no different from a similar group living on native diet. Whatever the cause of the high African S R, its clinical significance is probably an indication of diminished resistance to disease.

### SUMMARY

- 1 In Benue Province, Nigeria, 95 per cent of apparently normal Africans have abnormal S R, by European standards (normal = less than 5 mm in 1 hour using a Hawksley micro-tube)
- 2 Ninety per cent. of Africans with trypanosomiasis have abnormal S R s by African standards (normal = less than 5 mm in 10 minutes, Hawksley micro-tube)
- 3 Wide variations in the normal of different groups are found. The cause for this has not been determined but probably depends on differences in plasma proteins
- 4 The height of the S R in trypanosomiasis does not correspond exactly with the clinical condition but indicates the point of equilibrium between the patient's resistance and the virulence of the trypanosome. The S R does increase slowly as the disease progresses but with long stationary periods. A high S R may be due to a long-standing infection, a virulent recent infection, or concomitant disease. A low S R indicates a recent infection of low virulence
- 5 Women with trypanosomiasis have higher S R than men. Newly infected cases have higher rates than reinfected cases
- 6 Treated cases have sedimentation rates equal to those of normals in the same area
- 7 A pronounced fall of S R on administration of antrypol is diagnostic of trypanosomiasis

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## SERUM LIPIDES IN TROPICAL SPRUE

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From a biochemical standpoint, the special feature of the sprue syndrome is an impaired absorption of fat, which manifests itself by an increased amount of faecal fat. Direct measurement of the percentage fat absorption requires a fat balance experiment, which must be carried out over a prolonged period, if the results are not to be vitiated by irregular passage of stools. The fat balance technique is therefore ill-adapted to the study of short-term changes in fat absorption, and it was considered that estimation of the serum lipides before and after a fatty meal would supplement the results of stool analysis, and be more adapted to short-term experiments with possible therapeutic substances. BARKER and RHOADS (1937) have used blood fat curves to demonstrate improvement in fat absorption after liver therapy, and ADLERSBERG and SOBOTKA (1943) have also used blood fat estimations to support their claim that lecithin improves fat absorption in sprue. The present paper reports fat curves on a larger number of sprue patients than were available to these workers, it includes thirty-one curves on sixteen patients with tropical sprue, and twelve curves on nine normal subjects.

### MATERIAL AND METHODS

The normal controls were students or ambulant convalescent patients who had no disease involving the digestive system. The sprue patients were selected on the basis of severe steatorrhoea and loss of weight, all of them had acquired

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the disease on wartime tropical service in India or Burma, and the usual duration of symptoms was from 3 months to a year. Three of the patients had received liver treatment before their first fat curve; the others had had no therapy other than a low fat, high-protein diet.

The general procedure was to take a fasting sample of 10 ml. of blood, and samples at 2½, 3½ and 4 hours after the standard fatty meal. In some of the earlier curves, the timing of the samples was different, but experience showed that with the meal used the highest level of serum lipides lay between 2 and 4 hours, and the times given were then adhered to. The standard fatty meal consisted of tinned evaporated milk, and contained 18 grammes of milk fat. Most other workers have used a mixed meal of much higher fat content, about 50 grammes; we have deviated from the accepted procedure for the following reasons—

(1) Evaporated milk gives a homogeneous and accurately reproducible meal, so that it is possible to compare different curves in the same patient.

(2) The meal had to be tolerable even to the most severe cases of sprue; even with the small dose of fat used, several of our patients were nauseated, and they would certainly have reacted with larger meal.

(3) Larger meals give a more prolonged rise in the blood fats, which may not be maximal even at 4 hours; with our meal, the maximum rise fell within 4 hours, and the test could be completed in that time.

(4) All the patients studied were also on a fat balance, and it was desirable to give them a meal whose fat content was about the same as their normal breakfast, so that only small adjustments were needed in their fat intake for the rest of the day.

The standard fat meal was given by mouth, and not by duodenal tube, for it was found in preliminary experiments that fat given by duodenal tube produced less definite change in the chylomicron count and blood fat level than the same amount of fat given orally. This could be attributed to small intestine hurry when a quantity of material is introduced into the duodenum. It is of interest that PETERSON *et al.* (1947) found that sulphadiazine given by duodenal tube produced a lower blood level and urinary output than the same dose given by mouth.

Total fatty acid, lipide phosphorus and total cholesterol were determined on an alcohol-ether extract of 5 ml. of serum from each sample. For total fatty acids, the method of STODDARD and DREYER (1929) was modified by the use of a Jena sintered glass crucible, porosity 3, in place of a Gooch crucible and filter pad, in filtering the fatty acid suspension; the final titration was done in a 15 ml. long-necked volumetric flask, to minimize absorption of CO<sub>2</sub>. Lipide phosphorus was determined colorimetrically on ashed aliquots of alcohol-ether extract. Total cholesterol was estimated by Sackett's method (KING, 1945). Relative measurements of the opacity of the sera were made in a Klett-Summerson photo-electric colorimeter and the chylomicron count was also done (FOURMAN 1947).

## RESULTS.

The complete figures for the fat curves are given in the Appendix (page 251). The observed data in these tables are the total fatty acid in mEq./L., the lipide phosphorus in mg./100 ml., and the total cholesterol in mg./100 ml. The values given for phospholipide fatty acid and cholesterol ester fatty acid are calculated on the same assumptions as were made by PETERS and MAX (1943) in their extensive study of normal serum lipide values. The value given for

neutral fat fatty acid is the difference between the observed total fatty acid, and the sum of the calculated values for fatty acids in phospholipide and cholesterol esters

### THE FASTING VALUES

Table I gives the mean values and standard deviations of the lipid fractions in twelve fasting specimens from nine normal subjects, the values are in good accord with the much larger normal series of PETERS and MAN (1943). The sprue values given in the same table are derived from twenty-six specimens from thirteen patients, three patients who had received liver treatment have been excluded. The average value for total fatty acid is lower in sprue, but the difference is not significant. On the other hand, phospholipide and cholesterol fatty acids are both lower in the sprue patients, and the differences are significant ( $P < 0.02$  and  $< 0.01$  respectively). The average level of neutral fat fatty acid is higher in the sprue patients, although the difference

TABLE I  
FASTING SERUM LIPIDES IN NORMALS AND SPRUE PATIENTS

	Mean	Standard deviation	Coefficient of variation (Per cent.)
Normal (12 specimens)			
Total fatty acid (mEq/L.)	12.45	$\pm 2.47$	19.8
Phospholipide fatty acid (mEq/L.)	5.3	$\pm 0.78$	13.8
Cholesterol fatty acid (mEq/L.)	3.71	$\pm 0.54$	14.6
Neutral fat fatty acid (mEq/L.)	3.43	$\pm 1.83$	47.5
Ratio of phospholipide to total fatty acid	0.43	$\pm 0.04$	9.8
Ratio of cholesterol (mg/100 ml) to lipid phosphorus (mg/100 ml)	21.8	$\pm 2.14$	9.8
Sprue (26 specimens)			
Total fatty acid (mEq/L.)	11.05	$\pm 2.57$	21.5
Phospholipide fatty acid (mEq/L.)	4.64	$\pm 0.74$	16.0
Cholesterol fatty acid (mEq/L.)	3.10	$\pm 0.6$	19.4
Neutral fat fatty acid (mEq/L.)	4.10	$\pm 1.8$	42.0
Ratio of phospholipide to total fatty acid	0.397	$\pm 0.0557$	14.0
Ratio of cholesterol (mg/100 ml) to lipid phosphorus (mg/100 ml)	21.9	$\pm 4.15$	19.0

between it and our own small series of normals is not statistically significant, our mean level for neutral fatty acid in sprue is significantly higher than the mean level in the large normal series of PETERS and MAN ( $P < 0.02$ ). The ratio of phospholipide fatty acid to total fatty acid is significantly lower in sprue ( $P < 0.01$ ). The ratio of cholesterol to lipid phosphorus is the same as in normal subjects.

### CHANGES IN SERUM LIPIDES AFTER THE FAT MEAL

This analysis of the changes after a fatty meal is based not on "peak values," but on the average increment over the fasting value, using three specimens taken between 2 and 4 hours after the meal. It will be seen from the curves that the time of the peak after taking the meal is variable even in normal subjects, and a good general picture of the changes in serum lipides



cannot be based either on the highest recorded value (which may not be the true peak), or on a single fat estimation at a set time after the meal. The period 2 to 4 hours has been chosen because the highest recorded value for total fatty acids falls within that period in all the normal curves, and in all but two of the thirty-one sprue curves. Both in sprue patients and in normal controls, the highest recorded value was usually at 3 or 3½ hours.

Table II shows the average increment in total fatty acid and in the various lipide fractions, in eleven normal curves and thirteen curves on patients with sprue. From this sprue series, there have been excluded all

TABLE II  
AVERAGE FAT INCREMENTS IN NORMALS AND SPRUE

Subject	Total fatty acid (mEq/L.)	Phospholipide fatty acid (mEq/L.)	Cholesterol fatty acid (mEq/L.)	Mineral fat (mEq/L.)
		Normals (11 curves)		
1	0.27	0.06	0.06	0.25
2	0.26	0.11	—	0.40
3	0.30	0.04	0.07	0.30
4	0.36	0.09	0.06	0.25
5	0.40	0.21	—	0.41
6	0.44	0.12	0.13	0.79
7	0.52	0.17	0.16	0.83
8	0.51	0.24	0.09	0.88
9	0.55	0.30	—	0.79
10	0.67	0.44	—	0.81
11	0.79	0.54	0.41	1.43
Average normal increment		0.130	0.086	1.44
		Sprue (13 curves)		
1	0.46	0.11	0.23	0.2
2	0.41	0.11	0.21	—
3	0.46	0.09	0.04	0.34
4	0.29	0.10	0.00	0.43
5	0.11	0.06	0.01	0.1
6	0.21	0.21	0.2	0.20
7	0.21	0.07	0.04	0.46
8	0.41	0.05	0.06	0.57
9	0.43	0.23	0.1	0.60
10	0.46	0.04	0.00	0.4
11	0.03	0.06	0.03	0.44
12	0.13	0.21	0.21	0.21
13	0.41	0.06	0.07	0.32
Average sprue increment		0.073	0.036	1.2

The above table has been prepared by subtracting the fasting values from the values in three consecutive specimens taken between 2 and 4 hours after the meal. The differences have added and divided by three gives the average fat increment as reported in the table.

treated cases, and all the curves in which glycerophosphate or lecithin was given with the meal. In both normals and sprue patients, the change in all the blood values was variable after the meal. For total fatty acid one normal subject had a flat curve and two others had an average increment of less than 1 mEq/L. In the sprue series, one patient had no increase in total fatty acid, and seven others an increase of less than 1 mEq/L. The average increment in total fatty acid after the meal in the sprue patients was less than half that in the normals. Increase in phospholipide fatty acid was less constant than in total fatty acid—five subjects (normals) and six sprue patients showed a fall in lipide phosphorus after the meal. One factor in bringing about this variable

response in phospholipide fatty acid seems to be the fasting value of phospholipide, on the whole, curves with high fasting values are more likely to show a fall in phospholipide after the meal, but the correlation is not close. Since the fasting values for phospholipide were lower in sprue patients than in normals, there may have been some bias in favour of a phospholipide increase in sprue, but in spite of this, the average phospholipide increase in sprue cases was only 60 per cent of that in normal subjects. In the normal subjects, the meal caused very little change in the serum *cholesterol*, two of the subjects showed a slight fall in serum cholesterol, and the others small increases, the average rise in cholesterol-combined fatty acids being only 0.088 mEq/L. All but three of the thirteen sprue patients showed a fall in the serum cholesterol, the average decrease amounting to 0.262 mEq/L. As one would expect from the comparatively small changes in cholesterol and phospholipide fatty acids, the great part of the increase in serum lipides after the meal was due to fatty acid in the form of *neutral fat*. Thus applied both to normal subjects and sprue patients, but in the sprue patients the increment in neutral fat fatty acid was usually greater than the total fatty acid increment, owing to the fall in cholesterol fatty acid and often in phospholipide fatty acid. While the average increment in total fatty acid in the sprue patients was less than half that in normals, the increment in neutral fats was about 80 per cent of the normal increment. Only two of the sprue patients failed to show an increase in neutral fatty acid after the meal.

*In summary* in the normal subjects there was an increase in total fatty acids after the meal, a smaller and less constant increase in phospholipide fatty acid, little change in the cholesterol fatty acid, and an increase in neutral fatty acid sufficient to account for the greater part of the total fatty acid increase, the changes are in general accord with the results of fat curves in normal people as summarized by BLOOR (1943). In the sprue patients, the increase in total fatty acid was less than in normals, phospholipide fatty acid was also less increased, cholesterol fatty acids showed a definite fall, and the increase in neutral fatty acids was not significantly different from that in normal subjects.

#### CURVES WITH GLYCEROPHOSPHATE, CHOLINE, AND LECITHIN

VERZAR and LASZT (1934) showed that the addition of glycerophosphate increased the absorption of fat from intestinal loops in the rat. Preliminary experiments showed that the addition of 10 grammes of sodium glycerophosphate to our fat meal increased the chylomicron count both in normal subjects and sprue patients. Table III (page 246) shows the results of paired fat curves, with and without 10 grammes of sodium glycerophosphate, in one normal subject and six patients with sprue. In the normal subject, the glycerophosphate curve showed a greater increase in total fatty acid, mostly accounted for by an increased increment in neutral fat. Of the sprue patients, three whose fat curve without glycerophosphate was low showed an increase with glycerophosphate.

phosphate, and two others (Patients 1 and 6) whose fat curve was fairly normal, also showed a small increase with glycerophosphate. Patient 3 differed from the others in that his apparent fat absorption without glycerophosphate was unusually high, and he showed a smaller increment in the glycerophosphate curve than in the "control" curve. Glycerophosphate could not be said to have a specific effect on any individual fraction of the fat. For example, there were increases in phospholipide fatty acid increment in four curves, and falls in two—no very striking changes occurred in the cholesterol fatty acid, except in one curve where a large decrease in cholesterol fatty acid in the curve without glycerophosphate was absent in the glycerophosphate curve. Patients 2 and 5 who had shown a fall in neutral fat fatty acid in the curve without glycerophosphate, had increases in neutral fat fatty acid when glycerophosphate was given—but the other four patients showed smaller increments of neutral fat fatty acid in their glycerophosphate curves.

TABLE III  
FAT AND PHOSPHOLIPIDE ABSORPTION WITH AND WITHOUT 50 GRAMMES LECITHIN

Patient.	Total fatty acid (mg./L.)	Phospholipide fatty acid (mg./L.)	Cholesterol fatty acid (mg./L.)	Neutral fat fatty acid (mg./L.)
		Without		
1	1	4.1	— 22	8
2	4.4	4.9	— 21	3
3	19	10	— 0.43	0.23
4	— 21	23	— 0.56	0
		22	— 1	— 0.4
Normal curve	1.70	6.1	0.41	1.43
		With		
1	20	7.3	0.6	1.41
2	— 0.46	8.7	— 0.42	0
3	0.31	7.3	— 0.43	0.23
4	1.64	— 0.46	— 0.43	0.23
	— 0.4	30	— 0.47	2
Normal curve	1.2	3.1	0.43	1.75

ADLERSEBERG and SOBOTKA (1943) found that the addition of 10 to 15 grammes of 20 per cent. commercial lecithin to a fat meal increased absorption in a small number of normals and sprue patients—they used a single estimation of blood fat at 4 hours as their measure of fat absorption. In three curves done with and without lecithin, we used 10 grammes of a preparation found on analysis to contain 50 per cent. phospholipide, so that our dosage was a little higher than the actual amount of lecithin given by ADLERSEBERG and SOBOTKA. Of these three curves (Patients 5, 8 and 9) two showed a smaller increase in the total fatty acid when lecithin was given, while the third had a larger total fatty acid increase in the lecithin curve—but none of them showed the large increase reported by ADLERSEBERG and SOBOTKA. One patient (Patient 5), who had shown no increase in his lecithin curve, showed a definite increase in fat increment with glycerophosphate. The dose of lecithin used was small in

comparison with the glycerophosphate dosage, and it is possible that a larger dose of lecithin might have given a more definite response. Two curves done with 5 grammes of choline gave no evidence that choline increased fat absorption.

#### EFFECT OF LIVER TREATMENT

Table IV shows fat increments in four patients with severe sprue, before and after treatment with T.C.F., an Indian liver preparation. This was the only liver preparation available to us in adequate amount, in a dosage of 4 ml daily, it was found to be clinically effective, all the patients showing general improvement and definite gain in weight. The figures show that this clinical improvement was not attended by any dramatic improvement in the fat curve, on the contrary, three of the four patients showed smaller fat increments after treatment. These results are superficially at variance with those of BARKER and RHOADS (1937), who found that intensive liver treatment improved the blood fat curve, but they used a much larger fat meal, and a

TABLE IV  
AVERAGE FAT INCREMENTS BEFORE AND AFTER TREATMENT WITH T.C.F.

Patient	Total fatty acid (mEq/L.)	Phospholipide fatty acid (mEq/L.)	Cholesterol fatty acid (mEq/L.)	Neutral fat fatty acid (mEq/L.)
		Before		
4	0.20	— 0.10	— 0.26	0.45
10	1.48	— 0.04	— 0.06	1.50
11	1.07	— 0.52	— 0.19	0.54
12	0.13	— 0.03	— 0.35	0.52
		After		
4	0.23	— 0.17	— 0.03	0.43
10	— 0.19	— 0.16	— 0.10	0.02
11	0.80	— 0.29	— 0.07	0.57
12	0.40	— 0.09	— 0.12	0.61

different liver preparation, so the two sets of results are not strictly comparable. Although T.C.F. has been shown to be effective in pernicious anaemia, as well as clinically effective in sprue, it is not a "crude" liver preparation, and so may not have contained the unknown factor which improves fat absorption, as shown by BARKER and RHOADS. Our lack of improvement in the fat curves was in conformity with the failure of T.C.F. to diminish the steatorrhoea appreciably in these same patients.

#### DISCUSSION

It is reasonable to suppose that increase in the serum lipides after a fatty meal is mainly related to fat absorption. As a method of studying fat absorption, however, the use of blood fat curves has limitations which must be kept in mind in any interpretation. The change in blood fats is likely to reflect the rate at which fat is being absorbed rather than the total amount. For example, three

of the normal subjects had negligible changes in the serum lipides, yet none of them had steatorrhoea, so their ultimate absorption of fat must have been adequate. In the sprue patients, too, there was no close correlation between the increment in serum lipide, and the total fat absorption as determined by balance experiment. Even if fat is being absorbed at the same rate, the change in the blood fats may still be different from one curve to the next, for fat is removed from the blood-stream into the liver and tissue depots in a variable amount, at a rate which cannot be directly determined. The largest change in total fatty acid observed in our whole series was 5.3 mEq/L., and this could be accounted for by 10 grammes of absorbed fat. Most of the increments even in normal subjects were much smaller so it can be said that removal of fat from the blood occurs in considerable amount during the period of a fat curve and the observed blood changes are only the resultant of fat absorbed, and fat removed to the liver and depots. A further complication is introduced by the possibility of lipide shifts between red cells and serum. Considerations of this kind serve to explain the great variability observed by all workers on fat curves. The curves reported here are equally variable, and the "sprue" and "normal" ranges overlap widely. It can still be said, however that in sprue patients the average rise in serum lipides after a fat meal is lower than the average normal rise. This finding is in good accord with the more definite evidence of impaired fat absorption which is given by stool analysis. It confirms earlier work done on small numbers of patients (BARKER and RHODES, 1937; ADLERSBERG and BOBOTEA, 1943).

Comparison of the partition of lipides before and after the meal is of interest in relation to the hypothesis put forward by STANVUS (1942), to explain the mechanism of faulty absorption in sprue. STANVUS accepted the "partition theory" of fat absorption (FRAZER, 1940) which claims that fat is absorbed partly as "neutral fat" in a very fine emulsion, and partly as "split fat," in which case phosphorylation may be an intermediate stage in absorption. STANVUS suggested that in sprue the essential defect was in phosphorylation, and so the absorption defect would concern only split fat and cholesterol but would not affect the absorption of neutral fat. Those of our results which are in harmony with STANVUS's hypothesis may be summarized as follows:—

(1) The fasting values for cholesterol and lipide phosphorus are significantly lower in sprue patients than in normal subjects. The fasting values for neutral fatty acid are normal or even increased in sprue.

(2) After a fatty meal, the average increase in all the lipide fractions is smaller in sprue patients than in normal subjects but the neutral fat increment in sprue is 80 per cent. of the normal average, whereas the phospholipide increment is only 60 per cent. of the normal average. The fact that an increase in phospholipide occurs in about half the cases of sprue does not necessarily mean that they are absorbing fat in that form, for phosphorylation of fats also occurs in the liver and REINHARDT *et al* (1944) have shown that phospholipide

formed in the liver can enter the blood-stream, whereas phospholipide formed in the intestine is not available to the blood plasma. On the other hand, the results of blood analysis cannot exclude the possibility of fat being absorbed as phospholipide, and reconverted to neutral fat in the intestinal wall before entering the chyle.

Two of the sprue patients showed a fall in the neutral fatty acid after the meal. This might be caused by unusually rapid withdrawal of fats into the depots, but it is also possible that some cases of sprue may have an impaired absorption even of neutral fat, as a secondary phenomenon comparable to the general absorptive failure found in chronic starvation.

(3) Normal subjects show a negligible change in serum cholesterol after the fat meal, in spite of the fact that a fatty meal stimulates the outpouring of bile. In the sprue patients, the fatty meal is followed by a decrease in the serum cholesterol, which can best be explained by a failure to reabsorb the cholesterol secreted into the bile.

These findings can be interpreted as showing that neutral fat is well absorbed in most cases of sprue, whereas there is faulty absorption of cholesterol and of split fat, at any rate in the phosphorylated form. The general limitations of blood fat curves as a measure of fat absorption prevent these findings from constituting a satisfactory proof of the STANNUS hypothesis, such proof must depend on observing what goes on in the intestinal wall or lumen, rather than on such indirect inferences as may be made from the blood fat changes. These changes, however, lend themselves more readily to explanation by the STANNUS hypothesis than by any other at present available.

### SUMMARY

Total fatty acid, lipide phosphorus, and cholesterol were estimated in the serum of sixteen patients with tropical sprue, and nine normal subjects. These estimations were repeated at intervals after a standard meal containing 18 grammes of fat, from these data twelve fat curves in normal subjects, and thirty-one in patients with sprue, were plotted. It was found that the fasting level of total fatty acid in sprue did not differ significantly from normal values, but phospholipide and cholesterol were significantly lower, while the calculated value for neutral fat was higher than normal. After the fatty meal, the total fatty acids increased less in the sprue patients than in normal subjects, phospholipides showed a smaller increment than neutral fatty acid. The cholesterol, which was little affected by the meal in normal subjects, usually fell in the sprue patients. Sodium glycerophosphate, in a dose of 10 grammes, raised the height of the fat curve in five out of six patients with sprue, a similar effect was not observed with choline or lecithin. No significant change was demonstrated in the fat curve after a period of liver treatment in four patients.

Although the results of serial fat estimations in serum are no doubt affected by metabolic changes not directly concerned with fat absorption, the low fat

curve in sprue fits in well with the more direct evidence of faulty fat absorption given by stool analysis. The changes in the different lipid fractions are discussed in relation to STANTON's hypothesis that the absorption defect affects only those lipides which are phosphorylated during absorption. The observed results can be well explained on the basis of this hypothesis, but they cannot be said to contribute directly to proving it.

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## APPENDIX

RESULTS OF BLOOD FAT CURVES IN NORMALS AND SPRUE PATIENTS

The first five columns represent direct observation. The remaining three columns are calculated on assumptions described in the text. The figure for chylomicron count represents the actual count in a standard field that for opacity is the reading on a Klett Summerson photo-electric colorimeter with a red filter.

Time hr	Chylomicron count	Opacity	Total fatty acid (mEq/L.)	Lipide phosphorus (mg/100 ml.)	Total cholesterol (mg/100 ml.)	Phospholipide fatty acid (mEq/L.)	Cholesterol fatty acid (mEq/L.)	Neutral fatty acid (mEq/L.)
CURVES ON NORMALS WITH ORAL MEAL.								
(1)	0	2	—	10 75	8 0	4 64	2 73	3 38
	2	123	—	10 85	8 6	4 98	2 88	8 90
	3	90	—	10 85	0 7	5 02	2 95	7 05
	4	33	—	15 02	8 4	4 87	3 44	4 27
	5	10	—	12 58	8 5	4 93	2 43	4 64
				12 65				
(2)	0	3	29	10 55	7 5	4 35	2 08	3 22
	2	125	80	12 10	8 45	4 90	3 16	4 13
	3	115	112	13 23	7 8	4 53	3 07	5 03
	4	70	73	12 83	8 5	3 20	3 07	4 70
	5	60	48	11 83	6 4	3 71	2 96	4 90
(3)	0	1	35	13 45	0 25	5 37	3 46	4 08
	2	20	43	13 45	0 25	5 37	3 64	4 44
	3	20	43	13 45	9 3	5 30	3 04	4 42
	4	100	55	13 07	8 75	5 08	3 55	5 04
	5	40	74	14 70	8 5	5 02	3 48	0 20
(4)	0	15	32	10 80	8 5	4 03	3 63	2 33
	2	185	80	11 00	8 7	5 04	3 63	2 60
	3	140	03	11 08	8 45	5 04	3 50	3 44
	4	105	70	12 87	8 25	4 01	3 50	4 46
	5	20	79	12 74		4 78	3 50	
(5)	0	—	—	10 00	0 45	5 48	3 53	1 05
	2	—	—	11 22	8 75	5 07	3 70	2 46
	3	—	—	11 70	10 6	0 32	3 70	1 74
	4	—	—	11 83	8 6	4 03	3 70	3 20
	5	—	—	11 18	10 7	0 20	3 72	1 20
(6)	0	3	—	14 60	10 0	5 8	4 12	4 08
	1	5	—	14 74	11 5	0 07	4 12	3 85
	2	50	—	10 05	11 5	0 07	4 12	5 28
	3	50	—	22 02	10 4	0 04	4 12	12 40
	4	50	—	21 05	10 4	5 8	4 12	11 18
(7)	0	—	—	12 15	10 1	5 80	4 0	2 20
	2	—	—	12 40	0 45	5 48	4 0	2 02
	3	—	—	12 70	0 5	5 52	4 0	3 18
	4	—	—	12 15	0 5	5 77	4 0	2 03
	5	—	—	13 10	0 05		4 0	3 33
(8)	0	—	—	18 85	11 55	0 70	4 75	7 4
	2	—	—	15 15	10 65	0 18	4 67	7 74
	3	—	—	16 70	10 85	0 29	4 07	8 07
	4	—	—	10 10	10 95	0 36	4 07	7 91
	5	—	—	18 55	10 4	6 64	4 60	
CURVES ON NORMALS WITH DUODENAL TUBE MEAL.								
The numbers correspond to those already given as normal curves but with the same meal.								
(2)	0	3	—	8 02	7 3	4 23	3 74	0 95
	2	20	—	9 40	7 3	4 23	3 74	1 40
	3	15	—	9 40	7 8	4 23	8 94	1 20
	4	10	—	0 32	6 0	4 0	3 74	1 55
	5	10	—	0 25	6 0	4 0	3 74	1 51
(3)	0	5	—	12 31	10 0	6 15	4 42	2 74
	2	10	—	13 81	10 8	6 27	4 68	2 35
	3	20	—	14 25	11 45	0 64	4 08	2 98
	4	40	—	15 37	12 1	7 02	4 08	8 07
	5	30	—	18 60	11 8	0 84	4 42	2 94
NORMALS—WITH AND WITHOUT GLYCEROPHOSPHATE.								
The numbers correspond to those already given as normal curves but with the same meal.								
(9)	0	0	35	11 82	8 3	186	4 82	3 48
	2	80	52	11 82	8 4	186	4 87	3 48
	3	165	75	12 70	8 7	186	5 04	3 50
	4	60	84	12 78	8 8	186	5 04	3 48
	5	15	48	14 47		186	5 10	3 48
(10)	0	5	32	13 38	9 1	208	5 28	3 80
	2	150	108	14 82	9 45	208	5 49	3 80
	3	230	174	15 08	9 8	212	5 68	3 97
	4	140	159	16 00	9 85	208	5 72	3 89
	5	30	94	16 95	8 25	208	6 37	3 89



CHANGES IN SPRUE, WITH AND WITHOUT ELECTROPHORESIS.

Time hrs.	Cholo- sterol mg/100 ml.	Optical	Total serum fatty acid (mg/100 ml.)	Lipid- phosphorus (mg/100 ml.)	Total cholesterol (mg/100 ml.)	Phospholipid fatty acid (mg/100 ml.)	Cholesterol fatty acid (mg/100 ml.)	Neutral fatty acid (mg/100 ml.)
(1)				With-				
0	175.80	---	24	7.33	130	8.93	1.13	1.43
2	175.80	---	13.33	7.33	130	4.37	1.13	1.43
4	175.80	---	13.33	7.33	130	4.37	1.13	1.43
6	175.80	---	13.33	7.33	130	4.37	1.13	1.43
8	175.80	---	13.33	7.33	130	4.37	1.13	1.43
10	175.80	---	13.33	7.33	130	4.37	1.13	1.43
12	175.80	---	13.33	7.33	130	4.37	1.13	1.43
14	175.80	---	13.33	7.33	130	4.37	1.13	1.43
16	175.80	---	13.33	7.33	130	4.37	1.13	1.43
18	175.80	---	13.33	7.33	130	4.37	1.13	1.43
20	175.80	---	13.33	7.33	130	4.37	1.13	1.43
22	175.80	---	13.33	7.33	130	4.37	1.13	1.43
24	175.80	---	13.33	7.33	130	4.37	1.13	1.43
26	175.80	---	13.33	7.33	130	4.37	1.13	1.43
28	175.80	---	13.33	7.33	130	4.37	1.13	1.43
30	175.80	---	13.33	7.33	130	4.37	1.13	1.43
32	175.80	---	13.33	7.33	130	4.37	1.13	1.43
34	175.80	---	13.33	7.33	130	4.37	1.13	1.43
36	175.80	---	13.33	7.33	130	4.37	1.13	1.43
38	175.80	---	13.33	7.33	130	4.37	1.13	1.43
40	175.80	---	13.33	7.33	130	4.37	1.13	1.43
42	175.80	---	13.33	7.33	130	4.37	1.13	1.43
44	175.80	---	13.33	7.33	130	4.37	1.13	1.43
46	175.80	---	13.33	7.33	130	4.37	1.13	1.43
48	175.80	---	13.33	7.33	130	4.37	1.13	1.43
50	175.80	---	13.33	7.33	130	4.37	1.13	1.43
52	175.80	---	13.33	7.33	130	4.37	1.13	1.43
54	175.80	---	13.33	7.33	130	4.37	1.13	1.43
56	175.80	---	13.33	7.33	130	4.37	1.13	1.43
58	175.80	---	13.33	7.33	130	4.37	1.13	1.43
60	175.80	---	13.33	7.33	130	4.37	1.13	1.43
62	175.80	---	13.33	7.33	130	4.37	1.13	1.43
64	175.80	---	13.33	7.33	130	4.37	1.13	1.43
66	175.80	---	13.33	7.33	130	4.37	1.13	1.43
68	175.80	---	13.33	7.33	130	4.37	1.13	1.43
70	175.80	---	13.33	7.33	130	4.37	1.13	1.43
72	175.80	---	13.33	7.33	130	4.37	1.13	1.43
74	175.80	---	13.33	7.33	130	4.37	1.13	1.43
76	175.80	---	13.33	7.33	130	4.37	1.13	1.43
78	175.80	---	13.33	7.33	130	4.37	1.13	1.43
80	175.80	---	13.33	7.33	130	4.37	1.13	1.43
82	175.80	---	13.33	7.33	130	4.37	1.13	1.43
84	175.80	---	13.33	7.33	130	4.37	1.13	1.43
86	175.80	---	13.33	7.33	130	4.37	1.13	1.43
88	175.80	---	13.33	7.33	130	4.37	1.13	1.43
90	175.80	---	13.33	7.33	130	4.37	1.13	1.43
92	175.80	---	13.33	7.33	130	4.37	1.13	1.43
94	175.80	---	13.33	7.33	130	4.37	1.13	1.43
96	175.80	---	13.33	7.33	130	4.37	1.13	1.43
98	175.80	---	13.33	7.33	130	4.37	1.13	1.43
100	175.80	---	13.33	7.33	130	4.37	1.13	1.43

Time hrs	Chylo- micron count.	Opacity	Total fatty acid (mEq /L)	Lipide phosphorus (mg /100 ml)	Total cholesterol (mg /100 ml)	Phospholipide fatty acid (mEq /L)	Cholesterol fatty acid (mEq /L.)	Neutral fatty acid (mEq /L.)
CURVES ON SPRUE, WITH LECITHIN AND CHOLINE.								
Without								
(7) 0	8	—	8.25	6.3	110	3.05	2.22	2.38
2½	20	—	8.25	6.3	114	3.05	2.13	2.47
3	10	—	8.82	6.3	123	3.05	2.30	2.87
3½	5	—	8.82	6.1	119	3.54	2.22	3.06
4	5	—	8.08	6.1	121	3.54	2.26	2.88
With 5 grammes choline								
(7) 0	3	—	8.11	5.55	124	3.80	2.32	1.90
2½	40	—	7.75	5.75	117	3.34	2.10	2.25
3	50	—	7.00	6.1	113	3.54	2.11	2.31
3½	50	—	7.00	6.2	117	3.60	2.10	2.17
4	20	—	7.06	6.2	110	3.60	2.22	2.14
With 5 grammes choline								
(8) 0	58	—	12.38	8.35	175	4.84	3.28	4.28
2½	40	—	12.63	7.05	178	4.61	3.33	4.60
3	65	—	13.17	8.55	178	4.06	3.33	4.88
3½	43	—	13.17	8.50	178	4.03	3.33	4.01
4	44	—	12.30	8.10	178	4.70	3.33	4.27
With lecithin								
(8) 0	30	—	11.43	8.4	174	4.87	3.20	3.30
2½	97	—	11.43	8.4	168	4.87	3.15	3.41
3	83	—	11.22	8.4	171	4.87	3.20	3.15
3½	93	—	11.57	9.0	171	4.00	3.20	3.38
4	70	—	11.57	9.5	174	4.93	3.20	3.38
Without								
(9) 0	15	—	13.61	9.0	191	5.32	3.57	4.72
2½	70	—	14.47	9.0	180	5.32	3.37	5.78
3	83	—	13.83	9.05	183	6.35	3.42	5.08
3½	145	—	13.83	9.05	183	5.35	3.42	5.08
4	80	—	13.01	9.05	183	5.35	3.42	4.84
With lecithin								
(9) 0	5	—	13.50	8.05	184	5.02	3.44	5.04
2½	40	—	13.83	8.05	184	5.02	3.44	5.37
3	58	—	15.32	8.95	185	5.10	3.40	6.07
3½	133	—	15.02	8.75	184	5.03	3.44	6.50
4	85	—	16.13	8.75	184	5.03	3.44	6.61
With lecithin								
(5) 0	20	—	17.18	10.7	204	6.20	3.82	7.16
2½	195	—	17.18	19.7	190	6.20	3.72	7.26
3	90	—	16.89	10.3	190	5.97	3.72	7.20
3½	177	—	16.52	10.4	199	5.97	3.72	6.83
4	205	—	16.33	9.7	190	5.62	3.72	6.99
CURVES ON SPRUE BEFORE AND AFTER LIVER TREATMENT								
Before treatment.								
(10) 0	5	50	13.41	7.35	164	4.20	3.07	6.08
2½	60	77	13.06	7.05	153	4.03	2.08	6.0
3	45	98	15.84	7.2	164	4.18	3.07	8.50
3½	110	97	13.90	8.05	158	4.67	2.08	6.25
4	80	100	14.03	7.0	158	4.60	2.08	7.89
After treatment								
(10) 0	45	—	13.73	8.6	163	4.98	3.05	5.70
2½	15	—	13.80	7.75	159	4.40	2.08	6.33
3	70	—	13.01	8.25	150	4.78	2.08	6.25
3½	129	—	13.80	8.47	159	4.01	2.08	5.91
4	60	—	13.80	8.2	162	4.78	3.03	5.99
Before treatment								
(11) 0	—	—	11.00	6.95	177	3.97	1.31	3.81
2½	—	—	11.63	7.4	170	4.29	3.18	4.16
3	—	—	12.24	7.65	174	4.44	3.26	4.54
3½	—	—	11.84	7.75	176	4.40	3.20	4.00
4	—	—	12.25	7.9	176	4.53	3.20	4.46

Time hrs	Chole- sterol mg/100 ml	Openly	Total fatty acid (mEq/L)	Lipids phosphorus (mg/100 ml)	Total cholesterol (mg/100 ml)	Phospholipids fatty acid (mEq/L)	Cholesterol fatty acid (mEq/L)	Neutral fatty acid (mEq/L)
CURVE OF SPRUE BEFORE AND AFTER LIVER TREATMENT (continued).								
After treatment								
(11)	8	---	78.75	7.45	84	8.86	8.87	8.13
	21	---	11.44	7.1	100	8.86	8.86	8.93
	31	---	11.44	7.08	100	8.86	8.86	8.87
	41	---	11.44	8	102	8.86	8.86	8.86
	51	---	1.57	6.5	103	8.86	8.86	8.86
Before treatment								
(12)	21	22	10.74	6.54	101	8.77	8.43	8.81
	31	22	1.07	8	1	8.86	8.86	8.86
	41	22	10.74	6.54	100	8.86	8.86	8.86
After treatment								
(13)	21	---	11.12	7	103	8.86	8.86	8.86
	31	---	1.12	8	103	8.86	8.86	8.86
	41	---	1.12	8	103	8.86	8.86	8.86
	51	---	1.12	8	103	8.86	8.86	8.86
After treatment								
(14)	8	---	7.00	7.00	80	8.86	8.86	8.86
	21	---	7.00	7.00	107	8.86	8.86	8.86
	31	---	7.00	7.00	107	8.86	8.86	8.86
	41	---	7.00	7.00	107	8.86	8.86	8.86
See pre-treatment curves, see glycerophosphorus results.								
MISCELLANEOUS SPRUE CONTROL								
After liver treatment								
(15)	8	---	12.12	8.7	112	8.86	8.86	8.86
	21	---	12.12	8.7	112	8.86	8.86	8.86
	31	---	12.12	8.7	112	8.86	8.86	8.86
	41	---	12.12	8.7	112	8.86	8.86	8.86
Unselected								
(16)	8	---	12.12	8.7	112	8.86	8.86	8.86
	21	---	12.12	8.7	112	8.86	8.86	8.86
	31	---	12.12	8.7	112	8.86	8.86	8.86
	41	---	12.12	8.7	112	8.86	8.86	8.86
After liver treatment								
(17)	8	---	12.12	8.7	112	8.86	8.86	8.86
	21	---	12.12	8.7	112	8.86	8.86	8.86
	31	---	12.12	8.7	112	8.86	8.86	8.86
	41	---	12.12	8.7	112	8.86	8.86	8.86
After liver treatment								
(18)	8	---	12.12	8.7	112	8.86	8.86	8.86
	21	---	12.12	8.7	112	8.86	8.86	8.86
	31	---	12.12	8.7	112	8.86	8.86	8.86
	41	---	12.12	8.7	112	8.86	8.86	8.86
After days sometimes used and rabbits in								
(19)	8	---	10.45	7.75	77	8.86	8.86	8.86
	21	---	10.45	7.75	77	8.86	8.86	8.86
	31	---	10.45	7.75	77	8.86	8.86	8.86
	41	---	10.45	7.75	77	8.86	8.86	8.86
ADDITIONAL PORTING ALTHO SPRUE								
Numbers perturbed by those given in the curves								
Porting	5	---	10.71	8.82	84	8.86	8.86	8.86
	10	---	10.71	8.82	84	8.86	8.86	8.86
	15	---	10.71	8.82	84	8.86	8.86	8.86
	20	---	10.71	8.82	84	8.86	8.86	8.86
	25	---	10.71	8.82	84	8.86	8.86	8.86
	30	---	10.71	8.82	84	8.86	8.86	8.86
	35	---	10.71	8.82	84	8.86	8.86	8.86
	40	---	10.71	8.82	84	8.86	8.86	8.86
	45	---	10.71	8.82	84	8.86	8.86	8.86
	50	---	10.71	8.82	84	8.86	8.86	8.86
	55	---	10.71	8.82	84	8.86	8.86	8.86
	60	---	10.71	8.82	84	8.86	8.86	8.86
	65	---	10.71	8.82	84	8.86	8.86	8.86
	70	---	10.71	8.82	84	8.86	8.86	8.86
	75	---	10.71	8.82	84	8.86	8.86	8.86
	80	---	10.71	8.82	84	8.86	8.86	8.86
	85	---	10.71	8.82	84	8.86	8.86	8.86
	90	---	10.71	8.82	84	8.86	8.86	8.86
	95	---	10.71	8.82	84	8.86	8.86	8.86
	100	---	10.71	8.82	84	8.86	8.86	8.86

## TRANSITORY NEUROLOGICAL SIGNS IN SLEEPING SICKNESS

BY

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The appearance or development of symptoms indicating nervous involvement in sleeping sickness is somewhat rapid, usually taking a few or more weeks before becoming evident to the relatives and friends of the individual. As a rule, by the time the patient is removed to hospital it is not difficult to recognize that his personality has altered. His memory is not as good as previously, headache is troublesome, and he takes little real interest in himself, despite the fact that an initial anxiety for treatment may be evident. As the illness progresses he becomes more drowsy and tends to fall asleep at every opportunity. He is easily roused, but when awakened is likely to return to his previous state of stupor unless his attention to the centre of attraction is forcibly held. For a long time his habits are clean, but later he becomes incontinent of urine and perhaps faeces. Epileptiform seizures may occur at any period. His face becomes mask-like and there are commonly tremors of an irregular type in the face and hands, the tremor being especially noticeable when the patient brings his muscles into play. His speech is difficult to follow, becoming indistinct and staccato. He lies on his side either sleeping or with his eyes half open, staring vacantly into space. Even in the later stage, it is possible to get the patient to perform certain things or follow instructions, such as putting out his tongue, gripping your hand, and even to appreciate his sense of position. He will walk if told to, but with an unsteady and swaying gait and somewhat wide-based. His interest is maintained only for a short time and he readily lapses back into stupor. The appetite is poor. Usually the food lies at his side untouched, or perhaps he may gather sufficient energy to have one or two mouthfuls, or—as is more usual—he has to be fed.

\* My thanks are due to Dr R. M. MORRIS, Director of Medical Services, Southern Rhodesia, for his kind permission to publish this paper.

blood but without success. The enlargement of the glands in the neck only lasted a few days, after which they too disappeared. There was no enlarged gland elsewhere. Nothing was done for another 10 days when, as the patient was obviously becoming more stuporous another lumbar puncture was performed, and on this occasion it was reported that the cerebrospinal fluid was teeming with trypanosomes. The cell-count was 538 per c.c., probably all the cells being lymphocytes. The protein was high—86 mg per 100 c.c., and the chlorides were normal—720 mg per 100 c.c. The lumbar puncture was repeated 2 days later and the findings were confirmed, being in the main the same with sleeping sickness parasites still being seen in abundance. Further study as to the type of the trypanosome found was not made. The case, however, was probably of the Rhodesian type of sleeping sickness since Gambian sleeping sickness is not ordinarily found in the Rhodesias, and further the clinical picture—e.g., the almost complete absence of lymphatic glandular enlargement and the more rapid march of events—is suggestive of the former variety of the disease.

The patient was immediately put on to tryparsamide, together with a course of pentamidine (M. & B. 800). A total of 30 grammes of tryparsamide was given, 1½ grammes per week by intravenous injection and pentamidine in doses of 1 gramme daily for 10 days. The patient made very good progress: his weight increased, he felt stronger and began to take an interest in his surroundings. He became quite a useful aid in the ward in helping with sweeping and cleaning. His habits were now clean. By the time the course of tryparsamide had been completed, it was difficult to keep him any longer in hospital and he was repatriated to Northern Rhodesia. The last lumbar puncture done just prior to his discharge showed the protein to be 35 mg per 100 c.c. and the cell-count to be 24 cells per c.c. No trypanosomes were seen in the fluid.

#### SUMMARY

A case of sleeping sickness, probably Rhodesian, in which the patient developed transitory signs in the nervous system, viz. a focal palsy, extensor plantar response and unilateral optic neuritis is described. As it was not initially appreciated that these fleeting neurological phenomena may occur in the disease, its recognition was thereby rendered more difficult and was delayed.

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# THE INFLUENCE OF THE SUSPENDING FLUID ON THE SURVIVAL OF SPOROZOITES IN VITRO

BY

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*From the National Institute for Medical Research, London*

## INTRODUCTION

During the past few years many new compounds have been investigated for antimalarial activity, and attention has been especially directed to the effect of these compounds on sporozoite-induced infections, in the hope of finding a drug which would act as a true causal prophylactic.

In routine prophylactic tests in this laboratory, *Plasmodium gallinaceum* transmitted by *Aedes aegypti* mosquitoes is used as the test organism. The chicks are infected by the intravenous inoculation of ground-up heads and thoraces of mosquitoes known to contain sporozoites of *P. gallinaceum* in their salivary glands. Each chick receives the equivalent of one infected mosquito in a volume of 0.2 ml of suspending fluid.

In the early stages of this work it was noted that sporozoites suspended in saline tended to die off during the time taken to inject a series of chicks. In one experiment, when the number of chicks was large, the last birds in the series failed to become infected. Accordingly, an investigation directed to find the best medium for the suspension of sporozoites was carried out.

## METHOD

As in the routine tests, the heads and thoraces of a batch of infected mosquitoes were ground up in a small volume of the medium under test using a pestle and mortar, and the resulting suspension was washed out into a centrifuge tube. It was spun lightly to throw down major debris, and the supernatant drawn off and made up to volume, so that 0.2 ml of fluid contained

\* Acknowledgements are due to Miss R. BERSON for technical assistance

the equivalent of one infected mosquito. The same batch of mosquitoes was used throughout a single experiment, so that differences noted in subsequent injections were due to the suspending fluid and not to differences in the infection rate of the mosquitoes. The suspensions were allowed to stand for varying periods of time at room temperature (24 C.), and then tested for infectivity by the introduction of 0.2 ml aliquots into the jugular vein of clean chicks. Care was taken to mix the suspensions well before injection in order to obviate the possibility of settlement of the sporozoites to the bottom of the tube as a result of standing.

The results obtained in two typical experiments are shown in the table below.

TABLE

THE SURVIVAL OF SPOOROZITES OF *P. gallinaceum* IN DIFFERENT MEDIA 34° C.

Experiment number	Suspending fluid.	Time of standing hours.	Per cent. cells per osed on day					Number of chicks positive in group	Remarks.
			7	8	9	11	13		
I	Saline	0	2.8	25.7				3/3	Low infection in one chick 7th day <0.1 8th day
		0.5	<0.1	15.7				3/3	
		1.0	<0.1	<0.1	<0.1	<0.1		1/3	
		2.0	<0.1	<0.1		<0.1		0/3	
	Ringer's solution	0	0.1	45				3/3	Very low infection on 7th day reverting to <0.1 on 8th day with one exception
		0.5	0.1	<0.1	<0.1	<0.1		3/3	
		1.0	0.1	<0.1	1.7			3/3	
		2.0	0.13	<0.1		<0.1		3/3	
	Heparinized chick plasma	0	0.0	60				2/2	Heavy infection. Chicks died 7th-8th day
		0.5	0.7					3/3	
		1.0	9.5					3/3	
		2.0	7.5	70				3/3	
II	Heparinized chick plasma/ saline equill parts	0			34.3			2/2	
		10			22.4			3/3	
		13			10.3			3/3	
		14.0			11.4			3/2	

<0.1 = A few parasites seen in smears of one or more chicks. Average count remaining less than 0.1 per cent.

The sporozoites suspended in saline or Ringer all died off very rapidly, as shown by the irregular low-grade infections obtained in chicks by the injection of these materials half an hour after the suspensions had been made up. In contrast, sporozoites suspended in heparinized chick plasma showed no change in infectivity even after standing for 14.5 hours at room temperature.

Other experiments carried out showed that

1 The survival of sporozoites in citrated chick plasma was less good than that in heparinized chick plasma.

2 Reduction of the plasma concentration in saline below 25 per cent. results in poorer survival of sporozoites. Concentrations above this figure give the same results as the use of 100 per cent heparinized chick plasma.

3 The maximum survival time in equal parts of heparinized chick plasma and saline appeared to be about 18 hours, though the infection obtained in chicks after this period of time was delayed, showing that a high proportion of the sporozoites had died off. No infection of chicks was obtained by the injection of sporozoites which had been suspended in this medium for 24 hours.

4 The survival time of sporozoites at 37° C was much shorter than that at 24° C, a large proportion died or became non-infective after 4 hours at this temperature in heparinized chick plasma. In citrated plasma no infections were obtained after 4 hours at 37° C.

#### DISCUSSION

The fact that sporozoites survive for a longer time in plasma than in saline may be due to the presence of some nutrient substance in plasma, or it may be that physical conditions, e.g., colloid osmotic pressure, buffering, etc., are more satisfactory in plasma than in saline.

In similar experiments carried out by Mr P G SHUTE (private communication), using *P. vivax* transmitted by *Anopheles maculipennis*, infection was obtained with sporozoites which had been kept in Locke's fluid in sealed ampoules at 15° to 16° C for 48 hours. These results are at variance with those reported in this paper.

As a result of these experiments the routine prophylactic drug screening test has been modified so that a suspension fluid consisting of equal parts of heparinized chick plasma and saline is used in place of the saline previously employed. Using this method, regular infections have been obtained in all experiments carried out in the past 2 years involving the use of over 1,300 chicks.

#### SUMMARY

Experiments were carried out to find the best medium for the suspension of sporozoites of *Plasmodium gallinaceum* prior to injection into chicks, in connection with the technique in use for routine prophylactic tests on drugs.



Sporozoites survived for over 14 hours in equal parts of heparinized chick plasma and saline at 24° C. When Ringer's solution or saline was used as a suspending medium, few sporozoites survived as long as  $\frac{1}{2}$  to 1 hour at this temperature. At 37° C. survival times were much shorter in all the media tried.

A mixture of heparinized chick plasma and saline has been used as the suspending fluid in routine tests in this laboratory and regular infections have been produced in a large number of chicks.

## SUPPRESSIVE AND SCHIZONTICIDAL VALUE OF PALUDRINE (100 MG) IN VIVAX MALARIA

BY

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formerly Squadron Leader, Medical Specialist, R.A.F.V.R*

The following is an account of a series of twenty cases of benign tertian relapses treated with paludrine over a period of 6 months and followed for a further period of 6 months. The series is short as personal access to cases of malaria virtually ceased in June, 1946, it is reported as it is thought in spite of the shortness of the series, positive findings of value were obtained.

It has been stated (DAVEY, 1946) that "since a single dose of 100 mg of paludrine is enough to control a clinical attack of malaria so that a relapse does not occur for several weeks, the same dose given once each week is likely to be an efficient suppressive dose." FAIRLEY (1946) has stated that maximum schizonticidal effect might be expected from prolonged administration of small doses and it was considered likely that paludrine administered weekly in the above-mentioned dosage might prove more effective in eradicating the parasite than if given in larger doses for a shorter period.

I wish to thank Prof F J NATTRASS and Prof N HAMILTON FAIRLEY for their criticism and advice, the DIRECTOR GENERAL OF MEDICAL SERVICES, R.A.F., for permission to publish this paper, Dr DAVID CADDY, of the Royal Hospital, Portsmouth, for permission to publish details of the case which came under his care, and the Imperial Chemical Industries (Pharmaceuticals), Ltd, for the supply of paludrine.

*The patients* These were mainly ex-prisoners of war from the Far East who in every case had had previous attacks of malaria, varying from one to twenty in number and had had relapses varying in number from nil to ten since returning to this country.

*The method adopted* Each patient admitted for a malarial attack was treated with a single tablet of 100 mg. paludrine only. On discharge the patient was given twenty five paludrine tablets of 100 mg. each, with instructions to take one weekly. It is appreciated that some patients on this regime may not have taken their tablets regularly. In the cases later described, however there is every reason to believe that the patients who developed short and long-term relapses, had taken the course of tablets conscientiously.

A year after admission to hospital, i.e. 6 months after completing the course of paludrine, which itself had lasted 6 months, a questionnaire was sent to each patient. Information was especially sought in connection with long and short term relapses, the significance of which has recently been discussed by SHUTE (1946), and FAIRLEY (1947).

## RESULTS.

### 1. EFFECT OF 100 MG. PALUDRINE ON BENIGN TERTIAN MALARIAL ATTACKS.

During the period of acute illness due to a malarial relapse the response to a single dose of 100 mg. paludrine was marked by its slowness. In fifteen cases observed and so treated personally the temperature fell to and remained normal in 12 hours in only seven cases, while more than 48 hours was required in three cases. In a control series of cases of B.T. malaria in which quinine 10 grains t.i.d. was given for the first 3 days and mepacrine in graded doses for several days, the temperature fell to and remained normal in 12 hours in twelve out of fifteen cases—no case took more than 48 hours for the temperature to become normal. (See Table.)

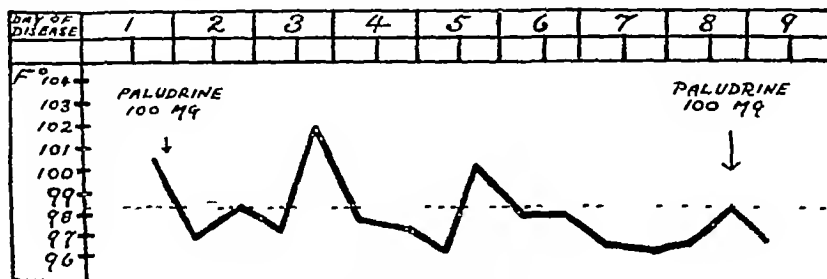
TABLE

		Single dose of Paludrine 100 mg.	Route quinine and mepacrine.
		Number of cases	
Temperature fell to and remained normal in	12 hours	7	12
Do	14 hours	3	1
Do	36 hours	1	1
Do	48 hours	1	1
Do	over 48 hours	3	

Case 1 illustrates the slow response to paludrine seen in three of the cases.

## CASE 1

The patient, a marine aged 24, had returned to this country in March, 1946, following a 2½ year tour of duty in India, Burma and China. He had been on suppressive mepacrine while in these parts, but ceased this on the journey home, and 3 weeks after so doing, developed his first attack of malaria. This was followed by a relapse in April, 1946, and the attack for which he came under observation by me developed on 14th June, 1946



Temperature Chart, Case 1

He gave a typical history of shivering, sweating, headaches, aches in limbs and anorexia, and on admission to hospital on 15th June, 1946, had a temperature of 100.5° F with B T trophozoites in the blood film. He was immediately given 100 mg paludrine. On the following day he was considerably better and the temperature normal, but a day later recommenced shivering, sweating, vomiting, had a temperature of 102° F (see chart), and the blood film showed B T trophozoites and gametocytes. No further antimalarial treatment was given and the next day he again felt well, but a slight recrudescence of his symptoms occurred on the following day with a temperature of 100° F. These cleared up within 24 hours and his further progress was uneventful, subsequent blood films being negative.

*Comment*

It is apparent that though paludrine in so small a quantity as a single dose of 100 mg controls the attack of vivax malaria, the process may be a slow one. The dose of 100 mg is probably sub-optimal and should be increased.

## 2. CONDITION OF THE PATIENTS DURING THE 6 MONTHS PERIOD OF PALUDRINE ADMINISTRATION

(a) No toxic manifestations of any sort were reported by the patients taking paludrine in doses of a single 100 mg tablet weekly.

(b) While having this dosage two patients reported having possible clinical malarial attacks manifested by shivering, headache and sweating, of sufficient intensity to make them stay off work for 1 or more days. A third stated that a day before he was due to take his weekly paludrine tablet, he had on several occasions a chilly sensation, with malaise, these symptoms were never of sufficient intensity to make him stay away from work, and they disappeared after taking the paludrine.

(c) One proven attack of malaria was encountered among the patients during the 6-month period of paludrine administration, the details of the case are as follows —

## CASE 2.

An airman, aged 24 had history of eight attacks of malaria in India, the last, in Delhi, was of the B.T. type and occurred in August, 1945. He returned from India in May 1946 and was admitted to hospital on 13th May 1946. He complained of malaise, headache, shivering and sweating, had temperature of 101° F and B.T. trophozoites were found in the blood. A single tablet of 100 mg. paludrine was given on the evening of 13th May and he responded slowly, pyrexia continuing till the morning of the 16th May, period of 60 hours. Thereafter he made an uninterrupted recovery and returned to full duty on 23rd May 1946. He was given 100 mg. paludrine weekly under the supervision of his medical officer and had taken dose on the 17th June 1946 but on the 20th June 1946 again developed symptoms of malaria, his temperature was 99.7° F and blood slide revealed very scanty B.T. mero. He was again given 100 mg. paludrine and the next morning the temperature had returned to normal.

No further attacks of malaria were reported during the subsequent 6 months in which paludrine administration to this patient was continued.

## Comment

The evidence suggests that paludrine in doses of 100 mg. weekly is occasionally insufficient to suppress vivax malarial infection since possible clinical attacks have been experienced in three, and an overt attack in one patient taking this dosage.

## 3. RESULTS OF A 6 MONTHS FOLLOW UP OF PATIENTS AFTER CESSATION OF THE COURSE OF PALUDRINE.

During the 6 months following cessation of paludrine therapy one proven and one doubtful clinical attack of malaria have been reported. The details of the proven case are as follows —

## CASE 3.

The patient, an airman aged 32, had been prisoner of war in Sumatra for 3½ years, during which time he had had numerous attacks of malaria. He was admitted to hospital on 29th April, 1946 with 48-hour history of malarial symptoms and temperature of 100° F. The blood film showed ring forms and scanty gametocytes of *Plasmodium vivax*. The liver was palpable on inspiration. He was given 100 mg. paludrine and the following morning the temperature was normal, but rose again in the evening, symptoms returned, and blood slide showed growing forms and gametocytes of *P. viv*. Thereafter he made an uninterrupted recovery, the temperature returning to normal 48 hours after the paludrine had been given. During the subsequent 6 months, while he was taking 100 mg. paludrine weekly he reported that he had had transient attacks of malaise with anorexia, slight shivering and aching of the limbs. He completed the course of paludrine in October 1946 and on the 21st February 1947, 250 days after his admission in May 1946, he was admitted to the Royal Hospital, Portsmouth, with symptoms of malaria, temperature of 101° F and sporoboid forms of *P. vivax* in the blood. He made an uninterrupted recovery from this attack and has been well since.

## Comment

It would appear from the evidence obtained in this case that paludrine at the dosage given cannot invariably be relied upon to prevent relapses of the long-term variety in B.T. malaria.

## SUMMARY AND CONCLUSIONS

1 In fifteen cases of benign tertian malaria treated with a single dose of 100 mg paludrine on 1 day only, the response was slow in comparison with that obtained following standard treatment for 3 days with quinine and followed by mepacrine. This single dosage of paludrine did, however, bring the attack under control.

2 No toxic manifestations were noted during the period of 6 months in which twenty patients were receiving 100 mg paludrine weekly. During this period, however, one proven short-term relapse of vivax malaria was encountered, and three possible clinical attacks were also reported.

3 During the 6 months period immediately following the 6 months of paludrine administration, one proven long-term and one possible clinical relapse were encountered.

These results suggest that a single dose of 100 mg paludrine is a sub-optimal dose in an acute attack. Doses of 100 mg weekly occasionally fail to keep the blood sufficiently free from parasites to prevent short-term relapses. This weekly dosage, continued for a period of 6 months, does not invariably prevent the occurrence of long-term relapses when the exhibition of the drug is discontinued.

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## CORRESPONDENCE.

## THE AETIOLOGY OF DESERT SORE

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I have just read Dr J. I. LESH's letter in the *TRANSACTIONS* (Vol. 40, page 906) commenting on my paper on the Aetiology of Desert Sore (*Ibid.*, 313). He is correct in describing me as an amateur in statistical methods, and my paper was written under conditions which did not allow me to obtain expert statistical advice. However the method suggested by Dr LESH namely to employ the standard error of difference of means to check the significance of the observed difference in healing times, was used but was discarded, as BRADFORD HILL (*Medical Statistics*, 3rd ed., page 78) states that this method is not suitable for small samples. The individual data, which Dr LESH would have liked, were not given in order to keep the paper within a reasonable length, but they are available.

I agree with Dr LESH as to some of the factors which may affect the healing of ulcers

(1) Virulence of organisms. The bacteriological investigations were as complete as possible in the conditions obtaining, and the bacteriology of both test and control groups, with the exception of one case of virulent diphtheria, was similar. Determination of the virulence of the commoner organisms, staphylococci and streptococci, was not carried out, and in the former was probably of no importance.

(2) The duration of the ulcers before treatment commenced was given.

(3) Area of ulcers before the onset of treatment were determined. These results were not published for lack of space. There was no significant difference in this respect between the test and control groups.

(4) The nature of the treatment employed is of course, dealt with in the paper.

It was appreciated, and made clear in the paper that the evidence put forward was inconclusive, and the suggestions as to the important factors in the aetiology of desert sore were described as tentative.

I am, etc.,

S. T. ARNOLD.

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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OPENING MEETING OF THE FORTY-FIRST SESSION OF THE SOCIETY

held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 19th October, 1947, at 7 30 p m

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P

in the Chair

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PRESIDENTIAL ADDRESS

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THE PRACTICE OF TROPICAL MEDICINE IN LONDON

BY

SIR PHILIP MANSON-BAHR

The successful practice of Medicine is founded upon  
a combination of knowledge, experience and judgment But the greatest of these is judgment

In justifying this title I shall endeavour to describe some practical experiences in consulting practice during a period of more than a quarter of a century here in London It is based upon an analysis of some 5,600 hospital records as well as probably almost as many again seen in consultation

At the outset it must not be imagined that the practice of tropical medicine in this country solely concerns itself with cases of tropical disease *sensu stricto*



Preferably it may be taken to represent that special kind of medical practice to be encountered in any tropical centre and comprises what might be termed rather a mixed bag. The cases discussed are tropical in so far as the majority have hailed from tropical or subtropical countries and include those for whose complaints some peculiarly tropical infection has been considered responsible, though in actual fact the connotation ranges over nearly the whole of medicine.

### MALARIA.

Malaria is still a common fever but only too often diagnosis is rendered obscure, or even sometimes impossible because of a universal inclination to ascribe any kind of fever to malaria the widely accepted notions regarding the persistence of these parasites, the frequent absence of any ascertainable physical signs such as splenomegaly the unreliability of laboratory methods of diagnosis during remissions, and the not uncommon habit of general practitioners of dosing patients with quinine or atabrin prior to seeking more expert advice.



CHART OF QUANTAN MALARIA.

There was no response to oral quinine but instantaneous response to intramuscular injection.

The patient, a seaman, had arrived from Durban and Beira. There was no splenomegaly and no parasites were found in the blood, after repeated examinations of thin and thick blood films during the rigors. No parasites were demonstrated in centrifuged citrated blood and the result of intravenous injection into a paralytic was negative.

Of one point I am certain never trust a home-made diagnosis, especially by one who senses malaria in his bones. I do not believe that it is possible on subjective sensations alone to differentiate one kind of rigor from another. As will be gathered in the sequel mistakes are most likely to be made with diseases of the genito-urinary tract, with diseases of the liver gall-bladder lungs or with the quite numerous blood dyscrasias.

I consider that the best and most reliable method of disentanglement from such a diagnostic dilemma consists in taking an accurate history with emphasis

upon the onset of the rigor and its effect upon general health. Sometimes it is true that a diagnosis has to be made upon the character of the temperature chart in the absence of demonstrable parasites in thin and thick films. I have treated two such cases of subtertian malaria and one of benign tertian from West Africa where intramuscular injections of quinine brought the fever to a full stop. In 1941, a seaman was in hospital with quartan rigors. Here there was no splenomegaly to act as a guide. Repeated blood examinations were negative, but again a quinine injection effected the desired result. (See Chart). These odd cases are probably much more frequent in this fever where quartan parasites are notably difficult to find.

*Superadded infection* may so modify the fever as to make the chart unrecognizable. It may be atypical, or lobar pneumonia. It may be syphilis.

In 1937 I treated an Australian planter from the New Hebrides. He was very ill indeed with subtertian malaria and no response to quinine or atebirin therapy was obtained until antisyphilitic measures were established. Punched-out ulcers (so-called "coral sores") on his legs were luetic and both Wassermann and Kahn reactions were positive.

#### SPLENOMEGALY

When is a spleen not a spleen? It may be an enlarged left kidney, hypernephroma or hypertrophied left lobe of the liver. The technique of splenic palpation is most important. For minor degrees of splenomegaly the right lateral position with the patient's left arm extended *well* above the head should be adopted. A splenic tumour usually moves freely with respiration whilst those of renal origin are more fixed.

#### HYPERNEPHROMA

Probably a not infrequent source of error is hypernephroma (Grawitz's tumour) which may be moveable and unassociated with renal symptoms, especially in patients with a previous malarial history. The obscure pyrexia (CATLIN *et al.*, 1947) which has also been noted by others, and which frequently heralds the earlier manifestations of this serious condition, does not make the problem any easier. Of this I had an experience in 1946 in a patient from Uganda in whom irregular pyrexia persisted for 3 months before the tumour became palpable, but by that time it had invaded the renal vein and the result was fatal. In this case no clue could be obtained from urinalysis.

*Pyonephrosis* may roughly resemble malaria. In 1927 a diagnostic puzzle presented itself in a young man from Guatemala who had been invalided for malaria unamenable to quinine therapy. For 2 years he had wandered up and down Harley Street seeking relief, but finding none. His chief complaint in addition to the rigors, was pain in the left hypochondrium where the so-called splenomegaly was found to consist of a much enlarged left kidney.

The urine contained a large quantity of pus and albumin. There was also localized pain in the renal triangle. At operation a large pronephrotic kidney was removed and was held to be congenital in origin.

#### PYELONEPHRITIS.

One of my most dramatic experiences was with a man of 48 from India who arrived by air (1932) on account of malaria associated with renal symptoms. He was so distressed that I feared he would die in my room. The anaemia was most severe (haemoglobin 30 per cent r.h.c. 1,995,000). He was oedematous, had retinal haemorrhages, enlarged and tender liver and spleen, polyuria, dysuria and pyrexia. The offensive urine contained numbers of *Bact coli*, pus and renal cells. He responded in a remarkable manner to blood transfusions and made a complete recovery on mandelic acid. He returned to India and retired in 1942. The splenomegaly mistakenly attributed to malaria was probably due to *Bact coli* septicæmia.

#### POLYCYSTIC DISEASE.

Polycystic disease of the left kidney may give rise to confusion as the lobulated character of the tumour may simulate the characteristic splenic notch.

In 1922 I was called to adjudicate on a medical officer who had for 18 years been in receipt of a pension for malaria and splenomegaly. That there was a large tumour in the hypochondrium was undoubtedly true, but careful examination of the urine revealing a low specific gravity, a trace of albumin and granular casts, led me to suspect that the hard, rubbery tumour was a polycystic kidney. He lost his kidney and his pension at the same time.

#### SARCOMA OF THE KIDNEY

The moral to be drawn is that in every case of suspected malaria on no account should a careful biochemical and microscopic examination of the urine be omitted, but this does not always avail. This possibility was demonstrated in a girl of 11 who presented herself with a much enlarged and painful "spleen" in 1930. She had suffered as a child from malaria in Bombay and the infection had manifested itself by relapses since her return to England. The issue was rather obscured by the story that she had fallen downstairs, seriously injuring her side, whereupon a large mass had revealed itself suggesting some intrasplenic haemorrhage. The urine contained an appreciable quantity of albumin, whilst blood examination revealed a moderate secondary anaemia and a slight leucocytosis. The tumour indeed in shape and character (including a so-called notch) simulated an enlarged spleen, and uroselectan did not shed any further light as both kidneys were functioning normally. Eventually the "spleen" was removed and proved to be an enlarged kidney (8) (b).

This was a congenital and cystic tumour with sarcomatous changes and was classified as *Wilm's tumour* (Diagram 1)

#### GASTRIC CARCINOMA

On anatomical grounds it is difficult to imagine a grossly displaced spleen and stories of this organ being found in the left iliac fossa should be regarded with suspicion. In 1936 a woman of 49 was referred to me with the suggestion of ptosis of the spleen as she had spent most of her life in India, and had suffered severely from malaria. She had been in England 4 months undergoing intensive atabrin treatment. She was therefore highly pigmented which did not make matters easier. The excessive weight loss aroused my suspicions,

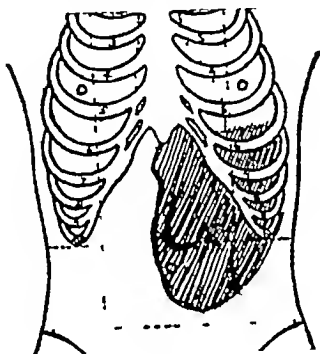


DIAGRAM 1  
Wilm's tumour of left kidney  
simulating enlarged spleen

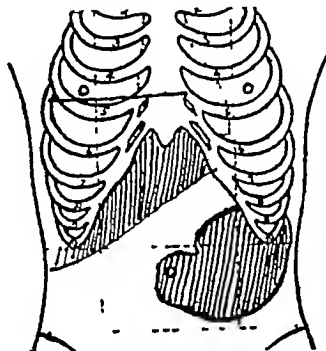


DIAGRAM 2  
Gastric carcinoma simulating  
displaced spleen

but the large, hard, moveable mass with a sharp edge (including a notch) jutting out at the level of the umbilicus and extending into the left iliac fossa did certainly resemble that organ, but at operation it proved to be an inoperable gastric carcinoma (Diagram 2)

#### HYPERTROPHY OF LEFT LOBE OF LIVER

Compensating hypertrophy of the left lobe ensues after partial destruction of the right. This was demonstrated in 1931 when I was called to a case of liver abscess which proved fatal. The tumour in the left hypochondrium which had been thought to be splenic turned out to be compensatory hypertrophy of the left hepatic lobe.

In a lady from Ceylon (1931) who had suffered from amoebic dysentery and had *Entamoeba histolytica* cysts in the faeces, a pyramidal shaped tumour in the left hypochondrium, which had been mistaken for a spleen, was proved to be an abscess of the left lobe of the liver.

## JAUNDICE.

Intense icterus of the billious remittent type of subtertian malaria may provide some diagnostic diversions. When it turns up in this country it is liable to be mistaken for biliary disease and recourse may be had to the surgeon's knife. This diagnosis is encouraged by the subhepatic tenderness so often elicited in acute subtertian malaria. In December 1944 a consulting engineer arrived by air after a 3 weeks' visit to the Gambia. Shortly after he suffered from fever with intense hepatic pain and jaundice. Biliary colic had occurred and he was said to have passed a calculus. As this suggested diagnosis was supported by a faulty cholecystogram, he was rushed into hospital for operation. But the spleen was palpable and the blood contained large numbers of subtertian trophozoites. The urine was stained green with bilirubin. The reaction to treatment was immediate.

A second case in 1946 was similar. He came from the Gold Coast and he too had been threatened with operation on the same ground but in this case the excuse was more plausible as there was no splenomegaly to act as a guide.

## CACHEXIA.

Malarial cachexia may be mistaken for Addison's disease on account of the anæmia, pigmentation and low blood pressure. In 1940 a young man arrived at Southampton in this condition from Dahomey and found himself in hospital labelled "Addison disease" and on corticoid treatment. I could find little excuse for this as his spleen was enlarged and there were subtertian parasites in the blood with profound blood changes.

## BLOOD DISEASES.

It is not surprising that acholuric jaundice should be at first regarded as malaria, especially when the patient has resided for a long period in an endemic area. There have been three of these, of which two were examples of the acquired type (Loritt and Morrison 1947). Two were in women. The first was a lady of 57 who, on account of enlarged liver and spleen with anaemia, had been finally diagnosed and treated as malaria and sent to England for a complete cure. Here the syndrome manifested itself in irregular pyrexia, anaemia, achlorhydria, retinal haemorrhages, a loud haemic systolic murmur, hepato- and splenomegaly. The serum contained 275 van den Bergh indirect units. There was increased cell fragility, a comparative leucopenia (4,000), but quite outstanding was the high persisting reticulocytosis (26 per cent). At operation the spleen weighed 8 lb. After an excellent recovery she returned to India and is still in good health.

The second similar case was in a woman of 35 from Kenya who had also been sent home for treatment of malaria. The correct diagnosis was suggested by a history of cholecystectomy 8 years earlier and pigmented gall-

PHILIP MANSON-BAHR

stones had been removed. The blood condition was identical with the above, but the reticulocytes were 18.4 per cent. Splenectomy (July, 1935) was successful. Three out of her five children, all boys, were found by Dr. JANET VAUGHAN to be subjects of this disease.

A third case in a young man of 23 (1938) from Calcutta had been diagnosed at various times as malaria, kala azar, paratyphoid, epidemic hepatitis, and pernicious anaemia. You could pay your money and take your choice!

There are also three records of splenic anaemia which have been subjected to splenectomy and which had been tentatively diagnosed as chronic malaria.

#### PYREXIA AND PERIODICITY

It is a commonplace that every kind of fever from the tropics is at first suspected as malaria and especially those with splenomegaly. Malignant endocarditis is a case in point and I find no fewer than eight cases of this in my records, but periodic fevers of the tertian or quartan type may be due to meningococcal septicaemia (PRIEST *et al.*, 1942, JAVETT and SACHS, 1942). In 1941 I was called in consultation to a Polish lady (aet. 50) who, fleeing from her native land, eventually reached Portugal in 1940 and was said to have contracted malaria there. In England the fever of a quartan type with facial oedema and urticaria was treated with quinine to which she was claimed to be allergic. I was impressed with the photophobia, neck rigidity and purpuric spots. Kernig's sign was absent. I umbar puncture established the diagnosis as cerebrospinal meningitis. She made a good recovery on sulphapyridine.

#### ISCHIORECTAL ABSCESS

Occasionally, in order to establish a diagnosis the most minute physical examination is essential and a small comparatively easily missed lesion may clear up a longstanding clinical mystery. In 1936 I was called to a naval hospital to examine an A.B. (aet. 35) who had spent 17 years in tropical waters. For a month he had high remittent pyrexia (never below 100° F. rising to 103 or 104° at 5 p.m.). Every conceivable laboratory test had proved negative except for a febrile albuminuria. On examining the perinaeum a minute para-anal swelling was revealed which on aspiration yielded a bead of pus containing TB. A small ischiorectal abscess was opened and the patient recovered. I possess also a record of tertian periodicity in apical pneumonia and also quite recently (1947) in a case of atypical virus pneumonia of the right lung in a patient from Sierra Leone who had been previously diagnosed on this account as subtertian malaria.

#### QUININE ABSCESS

In retrospect I must refer to a diagnostic digression to suppurating quinine abscesses simulating malarial pyrexia.

In 1932 it was my duty to examine an M.P. who 23 years previously had undergone an intensive course of intramuscular quinine injections in India. For months he had suffered from periodic rigors which had interfered with his activities in the House. In both buttock there were almond sized fibrous nodules. One had broken down and formed an abscess which was excised. Later in the same year a second instance occurred in a lady who had received similar quinine treatment 22 years previously in Malaya. Here a granuloma had formed at the site of injection resembling a sarcoma, and on section the tumour was found still to enclose crystal of quinine.

#### SUPRAORBITAL NEURALGIA.

The conscientious tropical consultant has to attempt to focus his watchful eye on every portion of the body and the nasal sinuses constitute no exception. Supraorbital neuralgia is not a frequent sequel of malaria of both tertian and subtertian types. When, however, cases with frontal sinusitis find their way to the consulting room they should be recognized and treated accordingly. As is well known the intense pain of frontal sinusitis usually comes after breakfast in the mornings and persists till the early afternoon—the clock-like rhythm constitutes an important point in diagnosis. This feature was borne upon me when I was consulted by a patient (1934) who had just arrived from the Cayman Islands (West Indies). He had been suspected of malaria on account of periodic attacks of pyrexia and supraorbital pain. I could find no signs of malaria and then remembered that in this particular group of islands malaria does not exist. On transillumination the left frontal sinus was opaque, whilst inspection of the nares revealed oedema, redness of the internasal septum and a purulent exudate in the middle meatus.

#### BLACKWATER FEVER.

As a rule when blackwater occurs in this country it does not give rise to any diagnostic difficulties. Though London may not be considered an ideal locality in which to conduct studies on this paradoxical question, nevertheless twenty-five cases have been investigated during the period under review, but the numbers have decreased since the introduction of atabrin. Fully half have been seen in what may be termed the pre-blackwater stage. It must not be imagined that blackwater arriving in England is mild in character for it can be exceptionally severe and rapidly fatal. I frankly admit that this hæmolytic may eventuate without any warning, nevertheless it is a clinical convenience to have some yard-stick to recognize the danger zone. I am guided by the cachectic appearance of the patient, the enlargement and tenderness of the spleen, the cherry-colour of the urine and by the presence of albumin. The importance of these signposts may be emphasized by the following experience—

One morning in March 1933 two Colonial officials recently arrived from the Gold Coast presented themselves for examination. Both exhibited the clinical phenomena outlined above and both had scanty subtertian trophozoites in the peripheral blood. I advised immediate hospital treatment. One, a police officer, who had just been decorated for meritorious conduct, refused all assistance as he was intent on watching a football match in Devonport. He was confident that he could treat himself with a teaspoonful of quinine. I was struck with his peculiar greyish yellow complexion and his rather rambling speech. It was a cold day with a bluing east wind. He saw his last game, blackwater supervened at 11 p.m. that night and he was dead 34 hours after during which period he passed only 4 oz. of urine. At autopsy he had acute necrosis of the liver. The other officer in hospital made an uneventful recovery on atabrin. That blackwater may ensue after a single infection with subtertian parasites under natural conditions was demonstrated in 1927 when I was called to a singularly acute case in Fleet Hants. The victim was a keen fisherman who was lured from home waters to fish for tarpon in the Caribbean Sea. He had landed for one night in Trinidad and returned to England in January of that year. During May and June whilst trout fishing on the River Test in Hampshire he suffered from periodic attacks of fever which were not recognized as malaria. In August (7 months after his return) he had a rigor and took quinine immediately blackwater developed and proved fatal. In August, 1922 the wife of a Colonial Office official arrived from Nigeria apparently in good health. Blackwater fever developed whilst she was attending a theatre and was so severe that she had universal haemorrhages with melæna. She died 9 hours after the onset and it was found that she had taken a large dose of quinine the previous night for a severe headache.

*Effects of Quinine* These and the following experiences left little doubt in my mind that in this acute haemolysis quinine constitutes a precipitating factor. This was illustrated rather tragically in a lady missionary from the Congo (November 1926) who had previously suffered from mild attacks of fever. On admission to hospital she presented the appearances of an acute subtertian infection of the bilious remittent type with numerous parasites in the blood. On the one hand she herself realized that she was in danger of dying from malaria; on the other she was equally convinced that she would succumb to blackwater if she was dosed with quinine. She was right! After an injection of 4 grains of quinine intramuscularly she died within 14 hours of blackwater.

My most sensational experience was in February, 1924 with an employee of Lever Brothers, who at the end of 3 months' leave in England was found to have subtertian malaria. Blackwater developed after he had been in bed for 10 days on quinine therapy. He had four separate haemolyses and the haemoglobinuria persisted for 14 days. When the anaemia was very severe (haemoglobin 10 per cent, erythrocytes 800,000) he jumped out of bed, hurled



the nurse downstairs, locked himself in and drank twelve bottles of beer. The mad escapade apparently saved his life! He was found drunk and unconscious on the floor and after this episode slowly recovered.

*Simulating Gastric Ulcer* The epigastric pain and vomiting which precede a blackwater attack may be mistaken for gastric ulcer.

Here is the case of a lady from Nigeria who, in March, 1925, was sent to England to consult a gastric specialist for persistent vomiting. During a barium meal she had a rigor and developed blackwater after taking 10 grains of quinine. This case was remarkable also because of a pyriform swelling which became visible in the epigastrium and which probably represented a distended gall bladder. During her critical illness amoebic dysentery and acute amoebic hepatitis made their appearance from all she made a good recovery.

### KALA AZAR.

Kala azar is not by any means frequent in European patients and therefore its advent to the consulting room is not usually anticipated. The difficulty is to suspect or recognize it at first sight. As a rule the appearance of the patient gives a different impression from that of chronic malaria. There is a white pallor rather than a sallow tinge. The eye has a different look—not quite the shining penetrating gaze of the malarial cachectic. The edge of the spleen imparts a hard and knobby sensation, and the splenic dullness extends above the costal margin. There may also be some oedema of the legs.

#### TUBERCULOSIS RESEMBLING KALA-AZAR.

The combination of hepatomegaly and splenomegaly may conjure up the vision of kala-azar in patients from a possible endemic area as in the following. In December 1933 I was consulted by an engineer who had lived in the Middle East and South Russia (Azerbaijan). He had suffered from an intermittent fever with splenomegaly and anaemia for which no adequate cause could be found. At first there were certain blood changes—a leucocytosis of 10,000 with high mononuclears (76 per cent), a chronic type of lymphadenoma was suspected. At the outbreak of war in 1939 he fled from France to England and 2 years later became very ill with high fever. Along with four other consultants I saw him in August, 1942, after a lapse of 9 years from the date of the first examination. It was then obvious that he was dying of generalized tuberculosis for I found a fistula in an exuding pus which was teeming with TB, whilst the faeces were also full of the organism. Here the splenomegaly and the blood picture were most probably also due to tuberculosis.

#### LYMPHADENOMA.

Any reticulosis with splenomegaly such as lymphadenoma may simulate kala-azar. At least eight such cases have been referred from this aspect to me.

during my career. We may also have to reckon again with hypernephroma as in the sad case I examined in 1944 in a young officer invalided from the Middle East. In this instance quite unnecessary diagnostic digressions had been caused by the reputed discovery of *E. histolytica* cysts in the faeces. The splenomegaly was due to left hypernephroma.

#### CARCINOMA

The converse may also happen and the diagnosis of lymphoma is often missed in this country. It may even be mistaken for carcinoma. In January 1945 I was called to examine an ex-Servicé patient (a/c 43) who had served in North Africa. After lengthy hospitalization in the Midlands he had been diagnosed as generalized carcinoma of the stomach and transferred to South London to be in the vicinity of his relatives as he was thought to be beyond all human aid. Shortly on admission his temperature and enormously swollen with ascitic fluidation was extreme; the temperature and leucocyte were reduced to 35.0 per centum. Diagnosis had been effected by blind puncture though the serum globulin test was negative. The liver and spleen occupied the whole abdomen and he was afebrile. His recovery was expected. Sulphonamide injections having had no effect he was given numerous blood transfusions without any appreciable result. Eventually he recovered with marvellous injections of subglucuronate though after the month an alarming allergic reaction nearly proved fatal.

#### AMOEBIASIS

Though amoebic dysentery is one of the commonest complaints for which patients apply to the tropical consultant this statement does not imply that it is by any means so universal as is popularly supposed. During the period under review there were over 600 cases in the old Hospital for Tropical Diseases under my care and this figure included only those which had been positively diagnosed by demonstration of *E. histolytica* or its cysts. As the result of this extensive experience of hospital cases as well as an unspecified number seen in consultation I feel that it would be advantageous to formulate some generalizations. This is rendered more desirable by a number of unauthorized wartime effusions which have clouded the issue.

(1) There exists a good deal of wishful thinking which tends to magnify the numbers of potential amoebic cases. Amoebiasis forms a convenient diagnosis satisfies the patient and therefore tends to be applied to almost any kind of intestinal disturbance for it should be remembered that there are many other affections of the large intestine which are accompanied by much the same phenomena.

(2) There still exists a wishful willingness to ascribe pathogenic properties to any species of amoeba found in the faeces.

(3) *Acute abdominal pain*, especially in the epigastrium or hypochondrium, and extreme meteorism are not features of intestinal amoebiasis.

(4) *Acute onset*, febrile attacks and vomiting are not characteristic of amoebic dysentery which is usually apyrexial. On the whole amoebic dysentery tends to be a chronic disease.

(5) Severe anaemia of the pernicious type is not an accompaniment of amoebic dysentery.

(6) As compared with bacillary dysentery tenesmus is infrequent, whilst loss of weight is not a marked feature.

(7) The presence of *E. histolytica* cysts in the faeces does not always completely account for the whole clinical picture as is amply demonstrated in this paper. Nor are they the cause of the anxiety neuroses so commonly met with. Of the amoeba it may well be said —

W seek him here  
 W seek him there,  
 W seek him everywhere  
 W may find him in heaven,  
 W may find him in hell,  
 Where you can find him, you never can tell,  
 That damned elusive pinpernel.

#### EFFECTS UPON HEALTH.

Amoebiasis of the bowel may persist for many years without causing a very noticeable disturbance.

A retired Indian official (æet 60) sought advice for a supposed rectal carcinoma. In 1910 he had suffered from amoebic dysentery and subsequently from liver abscess. After operation there was no recurrence of intestinal amoebiasis till 1941—a latent period of 31 years! He was then suffering from diarrhoea and tenesmus. Amoebic ulceration of the rectum was revealed by proctoscopy and *E. histolytica* cysts were numerous in the faeces. In 1939 a clergyman of 60 years was referred also as a possible carcinoma of the rectum on account of rectal pain, diarrhoea and dysenteric stools. It transpired that he had lived (1903 to 1923) in India and Iraq and suffered from dysentery there more than once. He had been in England 16 years. The same findings were present as in the previous case and he also made a good recovery. A third example was a Danish journalist (1931) who contracted amoebiasis in North Borneo in 1907 whilst on his honeymoon. This was his sole contact with the tropics. From that time onward for 24 years he had suffered from chronic diarrhoea and haemorrhoids. There was gross thickening of the colon with *E. histolytica* cysts in the faeces. He made an excellent recovery and when I last heard of him in 1939 he was suffering from constipation which was hailed as a great and welcome relief.

## MISTAKEN FOR ULCERATIVE COLITIS

Two cases had been subjected to appendicostomy as a method of treatment, as the result of a dubious diagnosis of ulcerative colitis but so far from relieving symptoms, the operation had tended to aggravate them.

In 1935 I was called to a London hospital to examine an elderly man who had been invalided from the South African war with dysentery and in 1901 this operation had been performed by an eminent surgeon for supposed ulcerative colitis. For 35 years his morning toilette had occupied 2½ hours as he was in the habit of washing himself out daily with 3 pints of water. In some blood stained mucus which exuded from the appendicostomy active *E. histolytica* were demonstrated. I am glad to report that he made an excellent recovery and the appendicostomy wound healed up.

In 1932 there was a somewhat similar case. This time in a man of 32 who had been invalided from Burma with amoebic dysentery which had persisted for the 9 years he had been resident in England. Treatment with emetine and EBI had failed so that this operation had been undertaken. Again active *E. histolytica* were found in the discharges. This time he responded to combined EBI and quinoxyl treatment and the appendicostomy was closed. I saw him several years afterwards. On the last visit he was suffering from renal calculi.

## DIFFERENTIAL DIAGNOSIS

## From Gallstones

It has fallen to my lot on several occasions to diagnose diverticulitis, polyposis polypi, and tuberculosis in cases suspected of amoebiasis and at other times the cause of abdominal pain has been found to be duodenal ulcer and cholecystitis gallstones and, most serious of all, carcinoma. As an example the following experience can be cited. The wife of a district officer from Northern Rhodesia (aet. 40) sought advice in December 1945. For 2 years she had recurrent attacks of agonizing pain coming on suddenly in the epigastrium. They were not to occur in the erect but whilst in the prone position. In 1944 the diagnosis of amoebiasis had been suggested by the discovery of *E. histolytica* cysts in the faeces but emetine therapy had not brought the desired relief. The diagnosis of gallstones was easily established by palpation of the gall bladder as well as by radiography.

## From Carcinoma

But a more serious error is to mistake the inequent symptoms of carcinoma of the colon or rectum for amoebiasis. Unfortunately it has been my experience to expose several of these tragedies. I well remember a fine naval captain (47) from Malta who for 2½ years had been treated as amoebiasis with emetine solely on the grounds that the faeces contained Charcot Leyden crystals. I

was evident that he had lost a great deal of weight. A fungating adenocarcinoma of the rectum was revealed by sigmoidoscopy just beyond reach of the examining finger.

*Double pathology* On the other hand rectal carcinoma and amoebiasis may coexist. I have seen three of these. I learned my first lesson in 1922 in a patient of 50 who returned to England from Calcutta with amoebic dysentery after 37 years residence. When *E. histolytica* in the active stage and its cysts were demonstrated in the faeces amoebic ulceration was recognized by sigmoidoscopy. Unfortunately a digital examination was omitted and a small malignant ulcer of the anal margin thereby missed. It should be emphasized that, in addition to sigmoidoscopy or proctoscopy a digital examination of the rectum is essential.

#### *From Lymphogranuloma*

Lymphogranuloma of the rectum or the ano-genital syndrome has also to be considered. In 1941 I was called in consultation to a woman in Surrey said to be suffering from amoebic dysentery with rectal stricture reputed to be the result of previous residence in South Africa and Malta. This was found to be an advanced case of lymphogranuloma with multiple fistulae of buttock and perinaeum and enlarged fibrous inguinal gland. The Frei reaction was positive. She died later of intestinal obstruction. This was undoubtedly an indigenous infection contracted in England.

#### HEPATIC AMOEBIASIS (LIVER ABSCESS) AND AMOEBIC HEPATITIS.

Amoebic hepatic abscess may be very easy to diagnose or it may be the reverse, for there is a type of case, and I have met them where every ancillary aid fails and the result has to be assessed by the unsatisfactory method of trial and error.

#### DIFFERENTIATION FROM CARCINOMA AND CHOLECYSTITIS.

Carcinoma may simulate hepatic abscess, as the deposition of secondary growths is often accompanied by pyrexia, rigors and night sweats. Sometimes, too, the demonstration of *E. histolytica* cysts in the faeces may divert the diagnostician. Then there is the differentiation of acute cholecystitis from amoebic hepatitis, in both of which hepatic tenderness may be localized over the gall bladder area. In acute cholecystitis tenderness is confined to the outer margin of the right rectus muscle. Murphy's sign is elicited and when referred pain is present, it is directed to the angle of the right scapula. The liver as a whole is not enlarged. Usually rigors are present and are accompanied by accentuated rises of temperature in place of the clock-like intermittent fever of hepatic abscess. The leucocytosis is higher, the direct van den Bergh reaction is raised and there may be deep jaundice.

## JAUNDICE

In liver abscess, on the other hand, *jaundice with bilirubinuria* is rare unless secondary infection with *Bact coli*, *Salmonella enteritidis*, haemolytic staphylococci or streptococci has occurred, or if there is direct pressure on the gall bladder, a most unusual event. Thus a difficult situation arises when the abscess is situated on the inferior surface of the liver in close proximity to the gall bladder.

I put forward this axiom: a high remittent fever with a temperature about 103° F with severe anaemia, gross enlargement of the liver and rapid wasting should always suggest a malignant growth, ascending pyelephlebitis or some other metastatic infection, such as actinomycosis, rather than liver abscess. Some of these difficulties may be appreciated by the brief recital of illustrative cases.

(1) *Abscess of the quadrate lobe*

A patient from Southern Rhodesia (aet. 58) 1925 operation for an acute appendix abscess. Subsequently repeated attacks of amoebic hepatitis. *E. histolytica* cysts found in faeces. In 1934 I treated him for chronic amoebiasis with EBI and quinoxyl. The liver was enlarged but otherwise there was no evidence of amoebiasis. In 1935 he was flown back to London with the diagnosis of cholecystitis (cholecystogram and localized pain). Operation by Sir JAMES WALTON, who, suspecting the latter, found instead a large abscess in the quadrate lobe. During convalescence, in spite of emetine injection therapy, amoebic dysentery intervened. On this occasion for some unknown reason it responded to treatment and he has remained well ever since.

(2) *Carcinoma may be engrafted on to a liver abscess*

(a) In 1936 an Indian seaman was admitted for loss of weight and hepatic pain. He was known to have amoebic dysentery and had recently been operated on for an abscess in the right lobe of the liver. Moreover, *E. histolytica* cysts were present in the faeces. Leucocytes numbered 18 000 and polymorphs 78 per cent. The fever was high and hectic. At operation numerous carcinomatous deposits were found in the liver apparently arising from the gall bladder bed.

(b) An ex-officer (61) who had suffered from amoebic dysentery in Gallipoli in 1915 and who subsequently had amoebic hepatitis was admitted (1931) with a history of recurrence of symptoms of 3 weeks' duration. *E. histolytica* cysts had been reported in the faeces. (Probably this was an error for *Endolimax nana* and *I. butschlii* cysts were actually present.) Bile was present in the urine. A large palpable mass in the right lobe of the liver proved at operation to be a carcinoma of the columnar cell type.

(3) *Hypernephroma*

*Grawitz's tumour* of the right kidney may produce a somewhat similar clinical picture. In 1934 a sad case occurred in a lady missionary from India who was admitted as having a liver abscess on account of *E. histolytica* cysts in the faeces. Symptoms had been present for 2 months. Suspicion was aroused by the presence of red blood corpuscles, leucocytes and albumin in the urine. At operation an enormous hypernephroma was revealed and this proved fatal.

(4) *Pseudohaemophilus hepatica*

*Pseudohaemophilus hepatica* (WINTER and BURTON, 1937a), a very rare disease (due to lack of blood fibrinogen), occurred in a nurse of 38 from Singapore. Her complaint was jaundice, clay-coloured stools and hydronephrosis (R), of congenital origin. *E. histolytica* cysts were found in the faeces. Her liver was enormously enlarged with a knobby surface. Biopsy showed marked perihepatic foci of round-cell infiltration and early periportal fibrosis.

(5) *Calcification of the Abscess Cavity*

On several occasions calcified hepatic abscesses, giving rise to no symptoms, have been revealed by radiography. In one a pensioner the calcified abscess was known to have persisted for 20 years. The abdominal pain of which he complained was found to be due to a duodenal ulcer. In another a woman of 73, a large calcified abscess was discovered 56 years after the original attack of amoebic dysentery during which time *E. histolytica* cysts had persisted in the faeces. During the whole of this period she had resided in England.

(6) *Girdle pain may be mistaken for hepatic pain*

(a) A woman of 35 who was born in Gibraltar and who had suffered from chronic dysentery at the age of 4 complained of right girdle pain and spasmodic contraction of the diaphragm with diarrhoea and anaemia. X-rays revealed an oval-shaped calcified abscess situated 1 inch from the spine between the 11th and 12th ribs at the level of 12th dorsal vertebra (stereoscopic method). It was aspirated by one surgeon who extracted 35 c.c. of pus. Eventually she was operated on when a cyst removed from the under surface of the liver and adherent to the diaphragm proved to be a calcified suprarenal cyst. (Plate Fig. 1)

(7) *Posterior root pain may be mistaken for hepatic pain*

(a) A Colonial official from Mauritius (æet. 25) was sent home as he had suffered from amoebic dysentery and had complained of constant right intercostal pain. *E. histolytica* cysts had been found in the faeces. I was impressed by his posture for he was bent forward with subcostal band-like pain. On examining his back the spines of the 7th and 8th dorsal vertebrae were prominent and tender. X-rays revealed the correct diagnosis as tuberculosis of the



1 Calcified suprarrenal (R) mistaken for calcified amoebic abscess of liver



FIG 2 Multiple basal-cell rodent ulcers resembling oriental sores from Northern Nigeria



FIG 3 Lymphatic cyst of axilla (cystic hygroma)



FIG 4 Myelosclerosis with erythroblastic anaemia. Referred as probable filariasis





bodies of the 7th and 8th dorsal vertebrae with calcified lesions at the apices of both lungs

(b) Another was a case of root pain caused by osteochondritis of the vertebrae. A war pensioner of 25 complained of pain in the right hepatic area. In the Middle East he had contracted amoebic dysentery for which he received treatment on several occasions. The pain was referred to the angle of the scapula and was aggravated by movement. X-rays of the spine revealed osteochondritis of the 7th and 8th dorsal vertebrae. There were Schmorl's nodes and definite narrowing of the intervertebral disc spaces.

### TROPICAL SPRUE

In former years a considerable portion of my practice consisted in the diagnosis and treatment of sprue. I have now collected records of 493 cases in hospital. These have been of all degrees of severity and the general impression left on my mind is that the disease has become far less frequent and less virulent in the last 10 years. At the present moment it appears to be in danger of extinction. With a malady of such obscure aetiology, mysterious in origin, so variable in its course and presenting such a strange combination of symptoms, it is not surprising that when it does arise after prolonged residence in this country the true nature is not recognized or that the syndrome is mistaken for something else. Thus I have had cases referred to me as gastric carcinoma, duodenal ulcer, cholecystitis, Addison's disease and pernicious anaemia. Some had even been operated on for gastric ulcer.

#### *Geographical Distribution*

Generally accepted beliefs regarding the limited distribution of this disease may have to be revised. My records show that I have diagnosed sprue from Egypt, Malta, Gibraltar and also from Southern Italy. With the exception of one doubtful case from the Sudan and a second from Nyasaland I have never seen a genuine example of sprue from tropical Africa. On the other hand, I have treated two men with sprue contracted in India who subsequently were sent in an official capacity to Nigeria with active symptoms of the disease. Both made an excellent recovery in that tropical climate and eventually reached positions of great responsibility in the State. Sprue may also disappear after recovery from some other acute infection. One of my patients contracted cholera in Persia and another recovered entirely after a singularly severe attack of pneumonia. One of the worst cases was in an Army officer I treated in 1930. He recovered and returned 7 years later with alcoholic neuritis. Although he was terribly ill then, he had no recurrence of sprue! The case from Italy (Naples) was rather a surprise. This was in an ex-soldier (*aet.* 23) who was found wandering in the streets in an advanced stage of the disease, having lost 70 lb in weight. The official military diagnosis had been "anxiety

neutrons and for 5 months in 1945 he had been subjected to psychological treatment. Previous to this he had been treated for amoebic dysentery. The correct diagnosis was patent. He was admitted to hospital and within 2 months had made an excellent recovery and eventually regained his normal weight.

#### OTHER MISTAKEN DIAGNOSES.

The main signs of sprue the glossitis, the loss of taste and smell which signalize its onset may lead the practitioner astray

#### *Antral Disease*

In 1940 I was summoned to a London hospital to examine a patient of 55 who had found his way to the ENT department as a case of antral disease. No improvement followed the customary washing out of the sinuses, but he soon developed a sore tongue and steatorrhoea so that the diagnosis of sprue became obvious. Recovery followed routine treatment during which he gained 14 lb in weight in as many days. When seen again 7 years later he had recovered. I think the mistake in this case was due to the fact that after leaving India he had resided 5 years in England before symptoms developed.

#### *Cholecystitis*

Sprue may be mistaken for cholecystitis as in the following instance. In 1941 I was called to a hospital in Essex to examine a woman of 59 who had formerly lived in Bombay but who had been in England for 16 years. Her illness had been a mystery. She had lost 64 lb. in weight and had almost the appearance of a skeleton. The significance of the sore tongue had been missed. A cholecystogram suggested cholecystitis and this, together with dyspepsia and the large pultaceous stools, had suggested the desirability of cholecystectomy and she was actually then being prepared for operation. A singular feature, in addition to the familiar signs of sprue, was alopecia. She made a wonderful recovery on the generally accepted liver treatment and when last seen in August 1942, presented a healthy appearance, rubicund and buxom. She was A.R.P. warden of her district. A pleasurable feature was the regrowth of the hair.

#### *Malignant Disease*

The sprue syndrome may be simulated by disease of the abdominal lymphatics. The first instance of this anomaly was seen in a young woman of 26 from Siam, who had been treated for sprue and at autopsy was found to have lymphosarcoma of the mesenteric glands.

A second case was in a man of 49 from Manila in 1929 who presented the classical features of the disease. He made a good recovery and returned to the Philippines. Nine years later he was readmitted to hospital with a severe



advancing granulomatous ulceration had persisted in the right groin. This was said to have occurred after an injury to the right knee which had resulted in an inguinal abscess. Contraction of the right thigh had ensued. The ulcer extended from the anterior superior spine and Poupart's ligament to 3 in. down the thigh into Scarpa's triangle. Superficially it somewhat resembled an ulcerating granuloma, but in the pus exuding from the surface tubercle bacilli were demonstrated. A skiagram of the thorax revealed an early tuberculous lesion at the apex of the right lung, whilst that of the spine gave the correct diagnosis as psoas abscess from an erosion of the body of the 5th lumbar vertebra.

#### TROPICAL ULCERS (*Ulcus tropicum*).

These are not frequent in Europeans under peace conditions, but when they do occur in healthy men it is impossible to associate them with any dietic deficiency. In 1933 I was consulted by a member of the Upper House who had contracted a deep tropical ulcer on the malleolus of the left ankle during a Mediterranean cruise. His chief complaint, apart from the pain, was his inability to pull on his hunting boot. The ulcer was deep and measured 2½ in. in diameter. It healed rapidly with iodoform powder and elastoplast strapping. The second case had a much more serious outcome. A district officer from the Benue Province of Nigeria came in August, 1934. Four months previously a tropical ulcer had formed on his right leg following a slight trauma. He was emaciated and exhausted by continuous fever as the ulcer had eaten through the muscles and periosteum, laying part of the tibia bare. Every kind of treatment proving ineffective, his leg had to be amputated.

#### DERMAL LEISHMANIASIS.

Many ulcerations are apt to be mistaken for dermal leishmaniasis. Perhaps one of the most noteworthy was a Colonial official from Northern Nigeria with multiple *rodent ulcers* in the face and neck (1937). The characteristic punched out appearance was typical and all cleared up with radium therapy (Plate Fig. 2, facing p. 284).

*Syphilitic ulceration* of the nasal septum and nares may closely resemble oriental sore. In 1944 a medical officer who had served in North and West Africa was sent to me for diagnosis. The history suggested leishmaniasis as a sequel to sandfly bites. Seven months afterward an ulcer with a glutinous scab had appeared. The Wassermann and Kahn reactions were positive and the ulcer healed with anti-syphilitic treatment.

result of sandfly bites. The sore was on the mentum, deeply excavated and surrounded by exuberant hairs. A sinus,  $\frac{1}{4}$  in in length, extended downwards to the bone. The suggestion that it might be due to an apical abscess of the lower incisor tooth was not well received. She returned  $4\frac{1}{2}$  years later and the sore was still there, if anything larger and more angry looking. A dental skiagram showed an apical abscess involving the roots of the two central and lateral incisors. There was no necrosis of the bone. The left lower incisor was extracted and during the operation a quantity of fluid exuded from the sinus, after which healing took place.

*An oriental sore* on the lower lip may closely resemble a syphilitic chancre and give rise to much heart-searching.

In August 1922 a young officer from Warizistan came home on leave and was sent to see me in a state of great perturbation. He had arranged to be married and was met at Southampton by his fiancée and a couple of maiden aunts who were horrified to behold a large indurated ulcer on the lower lip which had made its appearance on the voyage. His private doctor had whispered "chancre" as the submental gland was enlarged in consequence the patient was not permitted to kiss the lady of his choice. The situation was definitely eased by demonstrating leishmania.

#### LUPUS LIKE LESIONS

As has been established, 'apple-jelly' nodules may appear on the base of oriental sores which have responded to treatment. Their similarity to lupus lesions is great. Attempts to demonstrate leishmania fail, though these organisms can be recovered by cultural methods.

I have records of two such cases and, until I read ADLER's papers, I had suspected lupus. The first was in the daughter of an R A F officer from Baghdad (1938). The nodules had developed on the margins of a large healed oriental sore on the cheek. The lesions responded to Finsen light treatment. The second was in a Wren (*aet* 24) in 1944. In this case there was a good history of dermal leishmaniasis in Quetta 10 years previously. There was a healed ulcer on the left cheek with marginal "apple jelly" nodules.

#### LEPROSY

On three occasions patients have entered my room covered from head to foot with nodular leprosy which had erupted here in London. The first, a Belgian woman of 41 (November, 1922), told a distressing story. She was a mid-wife and had adopted this profession since the death of her husband 6 years previously. He was a Mauritian who had developed leprosy in this country and had obviously infected his wife. She succumbed to the disease 5 years later.

Leprosy may, of course, be extremely chronic in its course, and as in the

following case a latent period of over 30 years may elapse before the lesions become obvious.

In December 1942, a workman was referred to me by a medical officer of health. He was an old soldier who had served in India in 1899 and in South Africa for 6 years till 1905. In 1936 it was noticed that he had a florid seborrhoea of the face which did not respond to treatment and at the same time nodular lesions appeared on the eyelids and forehead. Quite noteworthy was the loss of the eyebrows and eyelashes and there were nodular lesions in both ears. Acid-fast bacilli were demonstrated in sebaceous excretion expressed from the nose and *Mycobacterium leprae* in a biopsy section of the skin of the cheek.

### HELMINTHIC INFECTIONS.

In European patients hookworm infections are usually symptomless and require concentration methods of the faeces for demonstration of the ova. Those with severe anaemia are rare. One was referred to me 5 years after the Armistice in 1918 as pernicious anaemia and here is the record of one where the infection had persisted years before giving rise to recognizable symptoms. A lady (1934), keenly interested in ornithology and entomology had spent some months in 1912 in the low country of Ceylon paddling through paddy fields. She had consulted several physicians for symptoms of low fever, lassitude and dyspepsia without result. Su-picion was roused by an eosinophilia of 12 per cent and numerous ancylostome eggs were found in the faeces.

#### CHLOROCHIASIS.

Three infections with *Clonorchis sinensis* are recorded, two of which were symptomless but there was one in a prominent Chinese politician (1936) the presentation of whose case caused a diagnostic headache. He had a lengthy history of cholecystitis with fever pain and finally jaundice. He was found to be infected with this fluke and so were the rest of his family. It was suggested that it might be the cause of a strawberry gall bladder with some calcification and pigmented stones as revealed by the cholecystogram. In addition he was a confirmed diabetic and operation was refused point-blank. Eventually he became the Japanese puppet at Nanking so that in retaining his gall bladder he lost his head.

#### BILHARZIASIS.

Infection with *Bilharzia mansoni* are mostly subclinical but this is the story of a patient (1938) sent home from Kenya to Cairo where the eggs of this parasite had been found in the faeces. The liver was enlarged and the hepatic pain was referred to the wing of the right scapula. I was struck with the greyish pigmentation of the face the ulceration of the sclerae and

the female distribution of the pubic hairs. The urine reduced Fehling and the fasting blood sugar stood at the level of 190 mg per 100 c.c. The main complaint which accounted for the hepatomegaly was haemochromatosis whilst the bilharzia constituted a purely coincidental infection.

### FILARIASIS

Any case with lymphatic obstruction is primarily considered to be due to *Wuchereria bancrofti*. The great majority are not. Thus a lymphatic cyst of the left axilla in a tea planter from Nyasaland (1936) was excised and found to be a *cystic hygroma* (Plate Fig 3). Aspiration had yielded a milky chylous fluid containing fat globules, but no microfilariae. Agents other than *W. bancrofti* may cause lymphatic obstruction. The exact replica of a filarial elephantiasis scroti was seen (1920) in a market gardener from Berkshire. Here the probable cause was chronic patches of psoriasis of both buttocks which had been treated daily with chrysarobin for 20 years.

*Elephantiasis nostras* exactly resembling in its course and manifestations filarial disease was seen in a Norwegian of 23 (1935). This involved the right leg and scrotum and was preceded by attacks of lymphangitis, the exact replica of filarial fever. He returned to his native land a happier man after operation.

### Epidemic Lymphangitis

An example of "epidemic lymphangitis" due to a haemolytic streptococcus presented itself in 1936 in an official from Antigua, Leeward Islands. This resulted from an injury to the little toe of his right foot. There was elephantiasis of the right leg. Apparently at that time similar cases had been observed on the island in a small epidemic. All filarial tests were negative. In the following year an almost parallel case was investigated in an Army officer (aet. 32) from Palestine. In this instance there was no history of injury, but the recurrent attacks of erysipelas and lymphangitis were most severe. For the next 8 years he was under observation till the introduction of sulphapyridine which controlled the attacks.

### Myelosclerosis

In 1937 a Swedish naval captain (aet. 57) was admitted as a possible case of filariasis. He had been sailing in tropical waters for 22 years and gradually symmetrical solid oedema of both forearms, sternomastoid region and scrotum had developed in association with a mild hypochromic anaemia. On X-ray examination dense sclerosis of the bones became apparent, especially in the pelvis and spinal column. The possibility of osteoplastic carcinomatosis was considered but eventually the designation "*myelosclerosis with leucoerythroblastic anaemia*" (WINTH and BRITTON, 1937b) was held to be more correct (Plate Fig 4 facing p. 284).



### Calabar Swellings

Infections with *Loa loa* are mainly distinguished by Calabar swellings but there are other allergic manifestations not mentioned in textbooks. Urticaria is one of these, but I have investigated three examples of a most irritating lichenoid eruption on chest and back accompanied by a high eosinophile leucocytosis. Biopsies of the skin have failed to demonstrate embryos of *O. rotundus*. Again I have records of allergic swellings with *Dipetalomonema perstans* but it is of course impossible to rule out a probable double infection with *L. loa*.

The fate of the *Loa loa* parasite in the body has always been a matter of interest to me. For a number of years I have had a lady from West Africa (Gaboon) under observation. She was found infected with embryos of *L. loa* in 1939 and suffered severely from Calabar swellings which persisted for 3 years. In 1945 she presented herself with numerous small painful cystic swellings over the body forearms, palms of the hands and fingers. The Calabar swellings had ceased and the microfilariae had vanished. On incision of the swellings cheesy necrotic material was expressed containing calcified remains of adult *L. loa*. There therefore appears to be some evidence that the death of the adult *L. loa* is associated in some way with the glands of Calabar swellings. This can be taken as yet another example of Parasites lost and Parasites regained.

## EYE COMPLICATIONS.

### (1) EYE COMPLICATIONS IN MALARIA AND BLACKWATER FEVER.

#### MALARIAL HEMIANOPSIA.

In October 1944 a district officer from Northern Nigeria arrived with hemianopsia of the right eye. He had suffered from subtertian malaria, but the blindness had supervened when in hospital suffering from rheumatic symptoms and in the absence of any evidence of malaria he received no specific treatment at that time. I found him to be heavily infected with *P. falciparum*. Ophthalmoscopy revealed the upper third of the disc as white and atrophic, and the atrophy had involved the papillo-macular bundle. The lesion was due to blockage of the superior branch of the central artery. Although the malaria infection cleared up rapidly with atebnin and quinine the altitudinal hemianopsia became permanent.

#### SUBHYALOID HAEMORRHAGE.

A large subhyaloid macular haemorrhage causing blindness of the right eye was seen in the wife of a district officer from Northern Nigeria in 1935. This came on after a mild attack of blackwater fever. Two years afterwards she was under my care again, this time with a second severe blackwater but without luckily any further extension of the haemorrhage.

## (2) RETINAL HAEMORRHAGE WITH ANTRAL SEPSIS

In 1942 I was requested to examine the blood of an engineer (aet 37) He had been the subject of extreme myopia since childhood and recently retinal haemorrhages had further obscured his vision. There was a microcytic secondary anaemia. In searching for a possible focus of infection the roots of the second upper molar tooth came under suspicion. In the dental X-ray part of the antrum was included, which showed pocketing between the first and second molars and dense opacity of the antrum and this, on transillumination, was opaque. Evacuation of 2 oz of offensive pus from the antrum resulted in the arrest of haemorrhage and consequent improvement of vision. (N B There had been no symptoms at all indicating antral disease)

(3) FILARIAL BLINDNESS (*Onchocerca volvulus*)

For 10 years a patient from Kenya has been under observation and opportunity had been afforded for watching the gradual progression of pannus until total blindness of both eyes had supervened. First infected in 1935 he has suffered from allergic manifestations, lichenoid skin eruptions, oedema of hands and feet and arthritis. Eye symptoms commenced 3 years later in England. Embryos of *O. volvulus* were demonstrated in skin biopsies over a period of 5 years (till July, 1943), after which they disappeared. It was noted that the pannus affected the portion of the globe exposed to the light so in 1944 the eyelids were stitched together by Mr A H Levy for 1 year without any evident improvement. In the following year the cornea of the right eye was removed and a corneal graft applied. This has been successful and the patient has now regained good vision in that eye.

## FINIS

I have now recounted a few of the ups and downs of consulting practice in tropical medicine in London. The way has led me along the bedrock of medicine and if I have strayed from the seductive path of pure scientific research, it has been because fate led me thither. I am not sorry, because by these means it may have contributed in some measure to the teaching of clinical medicine. There have been sad days and there have been joyful ones. There have been failures and some successes. Some of my patients have made light of their ills. "Give peace in our tum" said one, but "make and keep me clean within" rejoined another.

I am deeply indebted to my colleagues in the past especially Dr CARMICHAEL LOW, at the old Hospital for Tropical Diseases and also, to those of the Albert Dock Hospital

I could not have conducted such a practice on quasi-scientific lines for all these years had it not been for the constant, loyal and faithful assistance of Mr W J MUGGLETON FIMLEY who has been with me for so long.

Finally I would express the hope that some of the lessons here recorded and which we have learned together may be of some use to our successors in the difficult days which lie ahead.

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## COMMUNICATIONS

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### A RICKETTSIAL DISEASE IN EAST AFRICA TRANSMITTED BY TICKS (*RHIPICEPHALUS SIMUS* AND *HAEMAPHYSALIS LEACHI*)

BY

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The purpose of this paper is to describe a rickettsial disease occurring in East Africa and adjacent territories, with special reference to its epidemiology among Army personnel, the causative parasite the vectors and the morbid histology

In 1920 GILKS (1920) described a series of cases of a typhus-like disease in Kenya. This disease was not a new one, it had been known since 1914, and "for want of a better name" had been called "spotted fever" (ANDERSON, 1925). The history in nearly all

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\* We wish to express our thanks to Dr G L TIMMS, Medical Research Laboratory, Nairobi, for his invaluable assistance throughout this investigation, not only in the examination of many slides and the preparation of the histological material, but in many other ways, to Major E J LOWBURY, R A M C, for his co-operation and for his report on the Weil-Felix reaction and leucocyte counts, to Major A REEVES, R A M C, for his assistance in the field, and to Flt-Lieut BECK, D P M O, R A F, East Africa, for his co-operation, and to many others, especially Dr J H S GEAR, of the South African Institute of Medical Research, and Major VAN ROOYEN, R A M C. We are indebted to Lieut W J SHIELDS, D D E W (E W 2) and Sergt E J MORNEMENT, A F P U, who undertook the photographic work, and to B M DICK, who prepared the maps and graphs.

We acknowledge with thanks the assistance and encouragement of Brigadier E R CULLINAN, who suggested that this investigation be done, and the interest of Brigadier R P CORMACK, D M S, East African Command with whose permission this report is published.

cases described (GILES, 1920; CURRANKIN, 1921) had reference to an *exanth* (*tache rose*) fever, adenitis and macular or maculo-papular rash.

GILES (1927) suggested that tick might be the vector and considered that the disease was akin to Rocky Mountain spotted fever. All cases reported up to 1931 had occurred in the highlands of Kenya, in which year the first case was reported from Mombasa in the tropical coastal zone. JEWELL and CORSEACK (1930), in review of the disease in Kenya, were of the opinion that the vector was probably *imato*. This opinion was repeated by JEWELL and KAUFMAN (1932) and KAUFMAN (1933) suggested that rodents might act as a reservoir. The mortality in earlier reports of the disease is difficult to estimate, for while ANDERSON (1925) reported the "only fatal case so far known in the colony" JEWELL and CORSEACK (1930) stated that although the case mortality was at first thought to be nil, they were of the opinion that it was about 10 per cent. They qualified this statement, however, by saying that their figure "may be altered later."

The first demonstration of *Rickettsia* associated with human disease in East Africa was made in 1932 by TORREDO (1932). He injected guinea-pigs by various routes with an emulsion of "the entire metal lesion" from case of "tropical typhus." In certain of the injected animals he was able to demonstrate "acrotal reactions" and to carry out passage experiments. He stated that the rickettsiae which he had demonstrated were similar to the pictures published by MOORE (1925) and also by ANDERSON (1930). The vector and reservoir of the disease were, however, unknown. Further experiments (ROBERTS and TORREDO, 1933) incriminated *Rhipicephalus appendens* as the vector and ROBERTS (1936) described a "typical *septicemic* case" and in addition isolated strains of *Rickettsia* from fleas and rats.

Early in the East African military operations of World War II a mild typhus-like disease was observed among troops. These cases were few at first and did not present a military problem. This mild typhus-like disease was different from the occasional case of louse-borne typhus encountered among troops in Abyssinia. Sporadic cases were reported among H.M. Forces in many areas of Kenya Colony, the Somalilands, Abyssinia, Eritrea and the Anglo-Egyptian Sudan (Fig. 1). It was not, however, until 1944 that cases of the disease were reported in large numbers in epidemic form in the Mombasa coastal area.

After consultations on the identity of the disease, the possible vectors and the appropriate measures of control, we were appointed by Brigadier E. R. COLLIER, then Consulting Physician, East Africa Command, to conduct investigations directed to the solution of these problems.

We have therefore studied the distribution and seasonal incidence of the disease as shown in the Army and Air Force records and by our personal experience. We have made observations on ticks as vectors, and have examined tick infested houses, huts, and vegetation in areas frequented by persons who have become infected. We have recovered the causative rickettsial parasite and have reproduced the disease in guinea-pigs by inoculation of blood from patients, and with emulsions of macerated ticks. Our results although very incomplete, indicate that the disease belongs to the Rocky Mountain spotted fever group. Studies in cross-immunity, tissue cultures and natural transmissions by tick feeding are now being carried out. Results of these experiments will be submitted later. This preliminary account of our work is therefore in the nature of an interim report.

The clinical features of this disease in military personnel have been described by WALSH (1945), and in civilian cases occurring in East Africa by GILKS (1920) CLEARKEIN (1921), ROBERTS (1935 and 1939), LOEWENTHAL (1936) and SHELLEY (1943). From our study of the literature and from our discussions with district medical officers, we believe that the disease which we were dealing with in H M Forces and prisoners of war is the same as, or similar to,

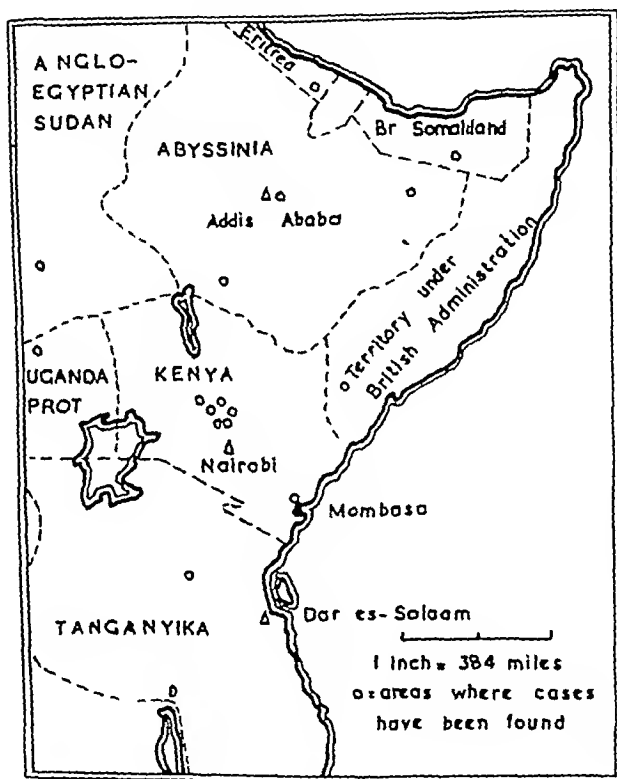


FIG 1

that which occurs sporadically throughout East Africa\* and adjacent territories

In the cases occurring among Army, R A F and prisoners of war personnel, a primary lesion or ulcer that developed into a characteristic eschar was found in no less than 81 per cent of patients (BOWLES, 1945). Lymphadenitis usually localized to the area draining the site of the eschar, local pain

\* East Africa is used to include Kenya, Tanganyika and Uganda

and pyrexia (see Chart I), were common symptoms. Some cases presented a macular rash of varying intensity and distribution, sometimes extending to the soles and palms. Headache and malaise are invariably recorded. The disease, in our experience, is mild and is always followed by complete recovery. In some instances, the exanthem is not found but we can find no valid reason for separating the disease into a type with initial lesion and another without one. Nor can we yet see any reason for sub-dividing the cases to include a severe form of the disease with pneumonic and myocardial involvements and splenic enlargement, and a milder form. These latter symptoms are, in our opinion, sequelae associated with constitutional defects and age. They are complications not directly concerned with the manifestations of the rickettsial disease *per se*. Much more investigational work is required, however, before attempting to explain the clinical variations hitherto recorded.

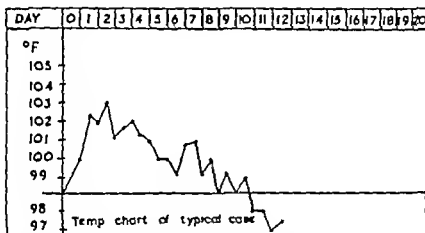


CHART I

LOGWENTHAL (1936) reported several cases of a typhus-like disease among the rural population of Teso in Uganda. He was of the opinion that the disease in Africans follows the same course as in Europeans. Although a very careful search was made for cases among Africans, only one case was found in a native soldier. The symptomatology and course of the disease in this African was in all essentials similar to that in Europeans. No cases have been found among Asian troops. The disease, with rare exceptions, is therefore apparently confined to Europeans.

### EPIDEMIOLOGY

When the disease was first notified in the Army medical officers acquainted with tick-bite fever of South Africa (GILK, 1938) identified the disease as such. Others, influenced by experience or reports of tick-borne typhus in Kenya

(ROBERTS, 1939), recorded the disease as "tick typhus," whereas many preferred not to commit themselves to an opinion nor to add to the existing confusion of names, and used merely general descriptive and convenient terms, such as "typhus-like syndrome," or "typhus syndrome." Probably more cases occurred than were notified, for in reviewing records in the *Army Medical Archives*, there are reports of cases with a history of an eschar, adenitis and a macular rash which were in all respects similar to cases diagnosed under one or more of the above terms. Army records show that this disease is widely distributed over East Africa (Fig 1). It has been reported from the Nairobi, Mombasa, Naivasha, Gilgil, Kijabe, Nyeri, Nanyuki and Eldoret areas in Kenya, from Mega, Addis Ababa, Babile and other unspecified areas in Abyssinia, from Mendera in British Somaliland, and from unspecified areas in Eritrea and the Anglo-Egyptian Sudan. Civilian cases have also been observed in other parts of Kenya, particularly in the Nakuru area (CHARTERS, 1945). At least two cases have been reported from Uganda (LOEWENTHAL, 1936), and some in rural districts of Tanganyika (SHELLEY, 1943).

The disease is not an urban one, nor is it restricted to townships (which it is necessary to explain may be equivalent to a hamlet, village or small town, with a considerable rural area, and containing numerous plots of undeveloped scrub). Certain of the military camps where sporadic cases of the disease occurred were situated within townships, but more usually outside on unoccupied land where the vegetation consisted of tall rank grass, scrub or trees, with a varying amount of shrubby undergrowth. Often the camps were many miles from townships.

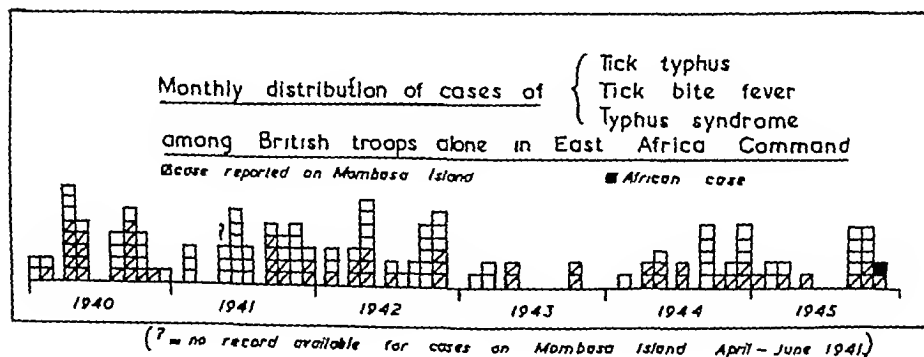


FIG 2

Fig 2 shows the number of cases among British troops alone, notified since 1940. As has been said, these cases were distributed over a wide area of the Command.

In 1944 an "epidemic" of this disease began in a prisoner of war camp





FIG. 4.—The type of country at the R.A.F. camps.

FIG. 5.—Pathway and huts, R.A.F. camp area, cleared of coarse vegetation.

FIG. 6.—Type of country surrounding P.O.W. camp before clearing.

FIG. 7.—P.O.W. camp, partially cleared—cutting and burning of grass has been carried out.

FIG. 8.—P.O.W. camp after complete clearing.

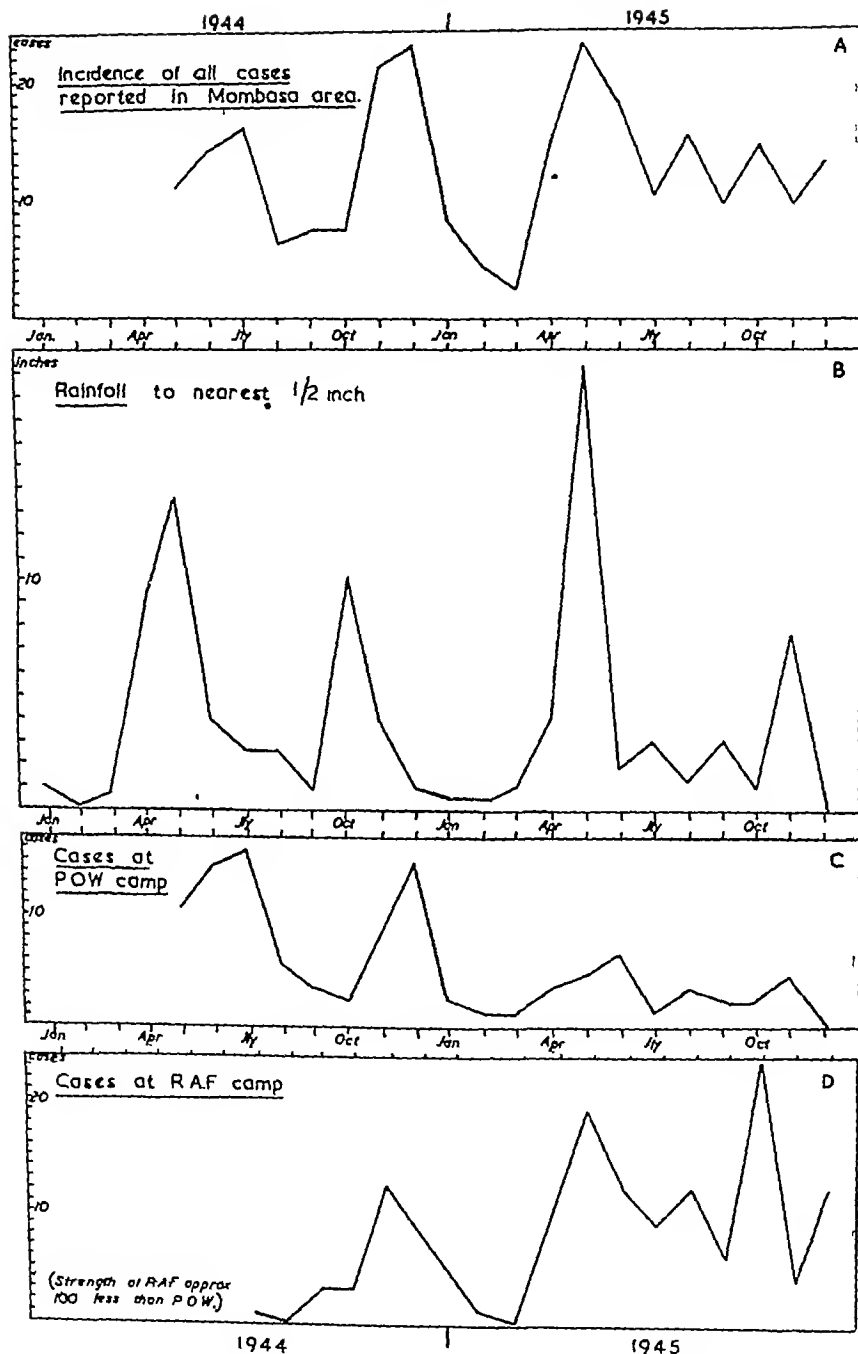


FIG 9

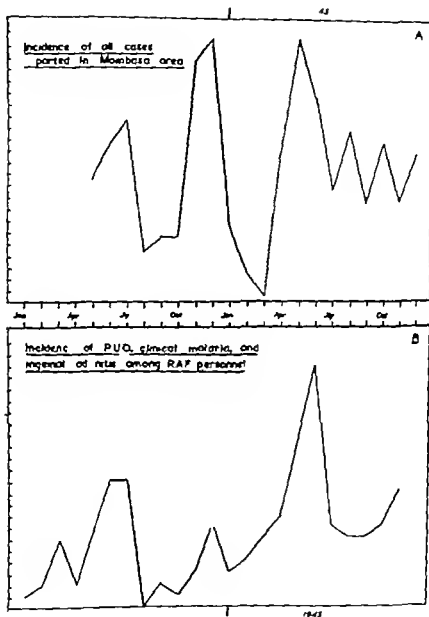


FIG. 10

were notified in areas other than the Port Reitz district, it does not mean that these soldiers were infected on Mombasa Island or forts, for it was possible that most of these cases acquired their infection in the Port Reitz area

Fig 10b is a graph showing the number of R.A.F. sick at Port Reitz who were classified as "pyrexia of unknown origin" (P.U.O.), "clinical malaria" and "inguinal adenitis". This graph runs nearly parallel to that of the entire epidemic and we believe that this is suggestive that many of these cases were probably mild forms of the rickettsial disease, similar to the *forme fruste* described in South African tick bite fever (PIPER and DAU, 1935, GEAR, 1938). The disease was probably more widely disseminated in the Port Reitz area than the actual notified cases would indicate, and it is possible that many subclinical and mild cases occurred.

ANDERSON (1925) had already called attention to the fact that in Kenya "pseudo- or para-typhus" appeared regularly every year at or about the time of the rains, but had shown no tendency towards assuming an epidemic form. TONKING (1932) observed that cases of "tropical typhus" were associated with the growth of long grass at the times of the rains. It can be seen (Fig 2, p. 299) that there is little variation in the number of cases from year to year among British troops, but that each year there are two maximum periods of incidence of the disease. These maximum periods of incidence correspond to the rainy seasons. Table II. This relationship is more clearly seen (Fig 9) in the Port

TABLE II  
NUMBER OF CASES AMONG BRITISH TROOPS ALONE ON MOMBASA ISLAND COMPARED WITH RAINFALL \*

Months	1940		1941		1942		1943		1944		1945	
	Rain-fall	Cases	Rain-fall	Cases	Rain-fall	Cases	Rain-fall	Cases	Rain-fall	Cases	Rain-fall	Cases
Jan - Mar	5	1	0	0	6	1	3	0	1	0	2	2
Apr - June	38	9	30	No record available	23	5	14	2	26	4	29	1
July - Sept	9	6	12	4	6	1	6	0	6	2	6	1
Oct - Dec	8	2	9	4	9	3	7	2	15	3	9	3

\* Rainfall recorded to the nearest inch (from R.A.F. Station, Port Reitz)

Reitz epidemic, where there is a very marked parallelism between the average monthly rainfall and the number of cases occurring per month. No relationship between the incidence of the disease and variations in temperature was found.

### PREVENTIVE MEASURES.

While we were naturally anxious to study the so-called epidemic at Port Reitz with the least possible disturbance of local conditions, it was obvious that an attempt at control was immediately necessary. We recommended —

(1) that all personnel be examined for lice, fleas, mites and ticks, and that all their kits be disinfected and disinsested

(2) that all huts and offices be carefully searched for possible vectors and that the huts be disinfected and disinsested

(3) that rats be exterminated (as far as possible) in and round the camps and any ectoparasites found on them be preserved for examination.

(4) that dogs be disposed of or destroyed after being de-ticked, etc.

We also recommended that the grass and any bush within the camp perimeters was to be cut short and burnt and maintained as short as practicable.

Additional preventive measures were taken with the prisoners of war whose movements were restricted. All long grass was put out of bounds to them and exercising outside the camp was restricted to 2½ miles of main road. Walking-out dress of slacks, boots and long stockings was made compulsory.

These preventive measures were put into action in December 1944. The cases among prisoners of war rapidly decreased. In March the restrictions on movements of prisoners of war were lifted but the cutting and burning of grass was rigidly maintained at their camp.†

Our recommendations were however more difficult to carry out at the R.A.F. camp. The incidence of cases among R.A.F. personnel increased during the rains at the end of 1944 and with the spring and autumn rains in 1945 (Fig. 9*d* p. 303). During this period the strength of the prisoner of war camp was about 100 greater than that of the R.A.F. camp. It may be argued that the marked fall in cases at the prisoner of war camp during and after the spring rains was due to the development of immunity among the prisoners of war. In our minds, however the difference in the two curves (Fig. 9*c* and *d*) is related to the proportion of preventive measures carried out, particularly to the amount of burning and cutting of grass at each camp.

Recorded at the R.A.F. Station, Port Reitz.

† This work was carried out under the supervision and direction of Major A. RAYNE, R.A.M.C.

During the entire period when these preventive measures were in force all insects were collected and forwarded to us for examination. No lice were found, fleas were few, no mites were found except three adult *Trombicula* which were collected in areas where no infection had been or was later recorded. Ticks, on the other hand, were abundant.

### TICKS

Some months after these preventive recommendations had been put into action we visited the area. We collected hundreds of ticks off the tips of long grass that had either not been cut or had grown on the sides of the roads and paths regularly used by the inhabitants of the camps. The predilection of ticks (adults and nymphs) for the long grass overhanging the paths and tracks is of special interest. From this grass the ticks could readily gain access to persons as they passed by and brushed against the infested tufts. (See Fig 11) During our survey of the area we found no parasitic stage of any mite, and although we ourselves were infested by ticks during our investigations we were not attacked by any biting insects other than mosquitoes. The clinical syndrome of the disease had long been associated in Kenya, Uganda and Tanganyika with tick bites. In the absence of evidence pointing



FIG 11 —Showing grass overhanging pathways where ticks, particularly *R. simus*, were found in great numbers, and readily brushed off on to passers-by.

to bites of other arthropods, our attention was concentrated on ticks as possible vectors of the disease. We were encouraged in this by the work of ROBERTS and TOWNSEND (1933) in Kenya of PIPER and GEAR and others in South Africa, and of other experienced observers in East Africa and elsewhere. Further we felt that there was a similarity between the disease and the tick-bite fever of South Africa. None of the patients was bitten by fleas, and our early experiment in the laboratory did not indicate that lice, fleas, or mites were vectors.

Further more early experiments using Egyptian gerbil (*Certhius gerbilus*) by Major LOWBURY which are said (CUTLER 1944) to show 100 per cent mortality when infected with blood from mite-borne typhus cases, had all proved negative.

A large variety of tick was collected by our colleagues and by us. The species included *Rhipicephalus simus*, *R. sanguineus*, *R. pulchellus*, *R. appendiculatus*, *Amblyomma variegatum*, *A. gemmum*, *A. lepidum* and *Haemaphysalis leachi*. The great majority of these ticks were adults. A fair number particularly those off dogs and cattle were nymphs. Larvae were extremely rare at the time of the survey. The most common species was *R. simus*. It is perhaps the most abundant and widely distributed species in the coastal belt of Kenya. This tick was slightly less numerous on dogs than *R. sanguineus* but on grass and on cattle *R. simus* predominated. The other species were relatively infrequent. In the hut of these camps only twenty-three adult ticks were collected. These consisted of fifteen *R. simus*, five *R. sanguineus* and three *R. pulchellus*. In the camp area *R. simus* was again the most numerous. *R. sanguineus* was not common either within the camp perimeters or in the scrub outside.

It is unnecessary to enlarge on the numbers and detailed distribution of these ticks. The points of interest in connection with the present subject may be summarized as —

(1) *R. simus* adults showed a marked predilection for tall grass overhanging path (Fig. 11), but was common everywhere.

(2) *R. sanguineus* was found chiefly on dogs prior to the institution of measures of control. Several specimens were afterward collected off grass, but they were fewer than the *R. simus*.

(3) No infestation of hut or offices, indicating that in some houses where dogs are kept was seen.

(4) Larval and nymphal ticks were rare when compared with adults and no clusters of larvae were found.

(5) *H. leachi* was present but not common.

(6) No ticks from rat or rat nests were recovered.

Ticks collected were separated into species, and again divided according to their phase of development, and whether fed or unfed. Each lot was macerated in saline, and inoculated intraperitoneally into guineapigs or gerbils (Egyptian and South African species—*Gerbilus gerbilus* and *Tatera brantsi*). Table III p 310, gives a summary of four successful transmission experiments. Using adult *R. simus* and *H. leachi* for maceration and inoculation into guineapigs, we were able to produce a disease in guineapigs identical with that produced by inoculation of blood from human cases. Two out of fourteen experiments using *R. simus*, and two out of nine experiments using *H. leachi* were positive. No positive results were obtained using larvae or nymphs of *R. simus* or *H. leachi*.

In guineapigs, after intraperitoneal inoculation of macerated infected ticks, the temperature rises between the 4th and 7th days (Chart II). The peak is usually reached on the 7th or 8th day. In none of our animals did the morning

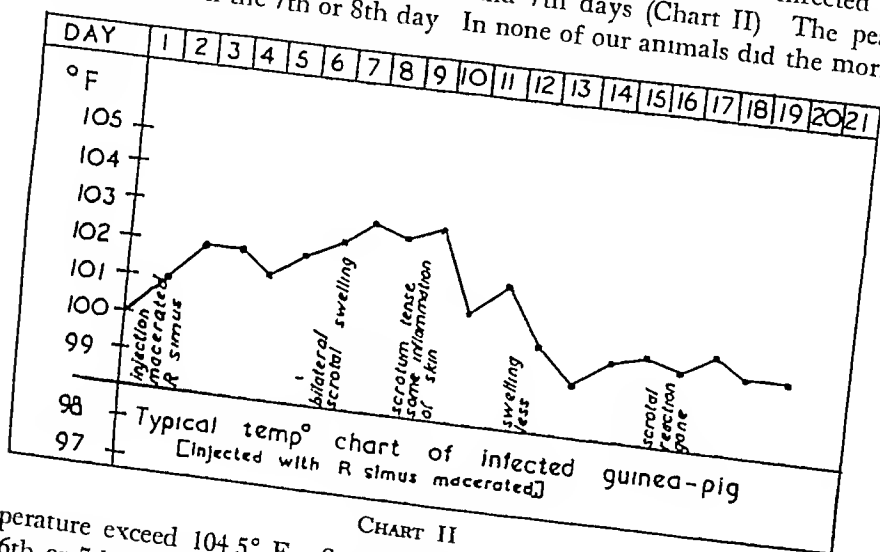


CHART II

temperature exceed 104.5° F. Scrotal swelling (Fig 12) usually appeared on the 6th or 7th day and was due to inflammatory changes in the scrotum, the tunica vaginalis, testes, adnexa and polar fat. In some cases no swelling was visible, but the scrotum on palpation had a tense feeling and the testes could not be pushed up into the abdominal cavity. The skin of the scrotum, in severe "reactors," presented a shiny appearance, and was oedematous. The tunica was grossly congested, and its visceral and parietal layers were some times glued together by loose sero fibrinous adhesions. Occasionally the tunica showed large haemorrhagic areas. No free fluid was found in the scrotal sac of any guineapigs except when ascites was present and this in all cases was associated with secondary infection. The spleen was seldom obviously enlarged but perisplenitis was not uncommon the capsule of the spleen having a milky



Number of experiment	Date	Ticks used for inoculation	Source of ticks	Material used for inoculation	Number of guinea-pig	Minimum temperature °F	Day on which maximum temperature reached
73	22.10.45	18 adult fed and unfed <i>H. leachi</i> macerated with acid in 10 c.c. of saline	Taken from dog in house where case was present (Nairobi)	2 c.c. of emulsified ticks	2A	104.8	4
76	28.10.45	4 adult <i>H. leachi</i> unfed	Taken from house where case of the disease had occurred 4 months previously (Nairobi)	6 c.c. of emulsified ticks	2A	103	8
81	5.11.45	9 adult <i>R. simus</i> unfed	Collected from the grass at the R. A. F. Camp Mombasa	8 c.c. of emulsified ticks	81A	102	4 & 5
					82A	100	2
83	22.12.45	1 unfed adult <i>R. simus</i> . Incubated for 40 min. at 37-38°C. and emulsified in 10 c.c. normal saline	Taken from L.A.C. E. between the fingers of the bird (Not attached)	2 c.c. of the emulsified tick	83A	103.8	4
					84A	100	2

## III

## TICKS FOR INOCULATING ANIMALS

Reaction of guinea pig	Fate of animal	Chief postmortem findings	<i>Rickettsia</i>	Passage experiments
On the 3rd day slight swelling, left scrotal etc. 4th day marked bilateral swelling. 5th day swelling decreased but area of inflammation over left testicle present	Used for tick transmission experiment on 4th day. Died on 6th day	Swelling of left testicle marked inflammation of left tunica vaginalis with moderate congestion of tunica on right side. Liver showed small areas of focal necrosis	++	The testes and adnexa, brain and spleen were emulsified in 30 c.c. of n. saline. 5 c.c. of this emulsion was injected into each of 2 S.A. gerbils and 2 male guinea pigs. The gerbils were observed for 31 days but showed no visible reactions whatsoever. One of the guinea pigs (10A) died of dysentery on the 3rd day. There was slight congestion of the tunica vaginalis and <i>Rickettsia</i> were demonstrated. The other (11A) had a temperature of 103.2° on the 4th day with typical scrotal reaction. <i>Rickettsia</i> were demonstrated and two successful passages were carried out.
Slight bilateral scrotal swelling on the 5th day	Sacrificed on 6th day	Generalized congestion of tunica vaginalis. A few hemorrhages beneath parietal tunica	+	No passage carried out
Slight bilateral scrotal swelling on 5th day	Sacrificed on 5th day	Slight congestion of tunica vaginalis. Perisplenitis present	Scanty	Testes and adnexa were emulsified in 10 c.c. of n. saline and 5 c.c. of the emulsion injected into guinea pig 70A. On the 6th day this animal showed a typical scrotal reaction and <i>Rickettsia</i> were demonstrated.
Nil	No abnormality noted up to 21st day	—	—	—
Moderate swelling and tenderness left side of scrotum on 5th day which became bilateral on 6th day	Sacrificed on 6th day	Congestion of tunica vaginalis particularly on left side	Scanty	No passage carried out
Nil	Died on 4th day with dysentery	—	—	—

coloration. Areas of focal necrosis were found from time to time in both liver and spleen. The mortality among infected animals was low although it was impossible to assess it accurately on account of outbreaks of dysentery among our animals. The guinea-pigs did not show any great loss in weight. The febrile reaction in guinea-pigs was of about the same duration as in humans. (Compare Charts I and II pp 298 and 309)

We cannot claim to have secured infection in guinea-pigs without difficulty. Our first 29 experiments, in which macerated ticks were injected into 123 guinea-pigs and 14 South African gerbils were entirely negative. Little purpose would be served by detailing these negative experiments. Suffice it to say that all experiments with fed and unfed larvae, nymphs and adult ticks of *R. putchellus* (five experiments), *R. appendiculatus* (three experiments) and *Amblyomma variegatum* (two experiments), *A. gemma* (one experiment), *A. lepidum* (one experiment), and *R. capensis* (one experiment) have been entirely negative. Tests with *R. sanguineus* (seven experiments) do not in our limited studies warrant, as yet, a definite pronouncement. We have succeeded in producing pyrexia in guinea-pigs with macerated *R. sanguineus* (Chart III) but obtained no acrotal reaction and we have failed to demonstrate rickettsiae and to carry out passage experiments using this tick for inoculation.

Our experiments of feeding clean adult ticks (*R. simus* H. Towns and *R. sanguineus*) on infected animals in order to test the infectibility of progeny are progressing.

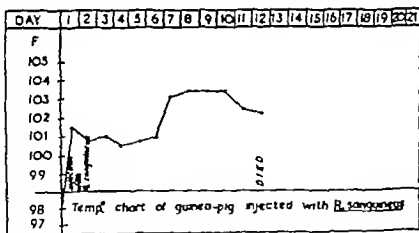


CHART III

### INFECTION OF GUINEAPIGS WITH BLOOD FROM PATIENTS.

Our early experiments in which we injected blood into guinea-pigs and gerbils, taken from patients at the height of pyrexia were un successful except



FIG. 12 —Guineapig, showing typical scrotal reaction (shiny appearance of scrotal skin)



FIG. 18.—Blood vessel showing endothelial proliferation (450)



FIG. 19.—Vessel showing strands of fibrin in which are polymorphs and cellular fragments (450)

for one (Exp 12 Table IV p 314) The lack of success in these experiments was due to the fact that we were apparently inoculating too small a quantity of blood into the animals. We employed whole blood, macerated clot and citrated blood for inoculation. The most successful results were obtained by directly inoculating guineapigs at the bedside with 6 to 10 cc of fresh blood. Using this technique in our later experiments we seldom failed to produce reactions in guineapigs demonstrate rickettsiae and carry out successful passage experiments. Guinea pigs injected intraperitoneally with blood from human patients and with macerated *R simus* reacted less severely than those injected with macerated *H leachi*. Similarly the reaction in passaged animals from originals injected with blood and *R simus* was less severe than in the *H leachi* experiments. No successful results were obtained by using any route other than the intraperitoneal route for inoculation.

In smears made from the tunica vaginalis of reacting guineapigs, parasites were found chiefly in large monocytes (Fig 13). An occasional neutrophil was also infected (Fig 14). In sections the intimal and smooth muscle cells of the media of some blood vessels contained rickettsiae which were more numerous than in smear preparations. The number of parasites was small, and varied from two to twenty, loosely distributed in the cell. They were never as abundant nor of the same morphology as in a preparation from a case of *sanguineus* borne typhus kindly put at our disposal by Dr ISCAER ROBERTS of the Medical Research Laboratory Kenya Colony (Fig 15). The cell pattern infection differed from that of ROBERTS (and presumably of 'ONKING), and more closely resembled that of "tick-bite fever" illustrated by GEAR and DOUTHWAITE (1938), GEAR (1938). The parasites, in our cases, were usually less numerous than in "tick-bite fever" of South Africa, and compared more with that of the "Eastern" strain of spotted fever illustrated by PINKERTON (1936). In our specimens, the organisms were usually confined to the cytoplasm but in several animals with a severe reaction, intranuclear forms were present, occasionally more than in the cytoplasm. They were nearly always lanceolate and diplo-bacillary (Figs 14 to 16). The morphology of the rickettsial pattern of cell infection and type of cell involved has been similar in our experiments with blood, *R simus* and *H leachi*. Rickettsiae were more numerous and more cells were infected in our *H leachi* experiments.

#### PATHOLOGICAL CHANGES

In sections of the tunica, testes and scrotal skin, the most marked pathological changes were observed in the blood vessels. Most of the vessels showed endothelial proliferation (Fig 18) with hyaline degeneration of the heaped up cells. Some of the vessels contained loose strands of fibrin (Fig 19) in which lay cellular fragments and many polymorphs. Other blood vessels contained thrombi, consisting of fibrin, degenerated white cells and hyalinized debris.

TABLE  
SUCCESSFUL EXPERIMENTS USING BLOOD FROM

Number of experiment.	Date	Source of material used for injections of each animal. †	Number of guinea-pigs	Maximum temperature. °F	Day maximum temperature reached.	Reaction of guinea-pig
12	18.12.44	Injected with 8 c.c. of curried blood from case in Mombasa on the 4th day of illness by E.J.L.	ML10	101	12	Nd
			ML11	100.8	7 & 8	On 7th day some inflammation of scrotum on right side and swelling of right testis. Disappeared on 8th day
			ML13	102	10	On 8th, 9th and 10th days bilateral swelling of scrotum and testes.
40	21.12.44	10 c.c. of blood taken at the bedside from patient in the early febrile period of illness and injected immediately into guinea-pigs.	84	101.8	3	Nd
			85	102.8	8	Mixed bilateral scrotal reaction on 8th day
			86	102	4	Oedema of scrotum and tense feeling of scrotum and testes on palpation on 8th day
72	22.10.48	10 c.c. of curried blood from patient on the 3rd day of pyrexia (C.M.L. Patient referred Nairobi)	1A	100	6	Slight swelling of scrotum on 8th day
			2A	99.8	4	Nd

Temperatures are recorded at 05.0 hours daily

† All animals were injected by the intraperitoneal route

## IV

## INFECTED PERSONS FOR INOCULATION OF ANIMALS

Fate of guineapig	Chief postmortem findings	<i>Rickettsia</i> present in smears from tunica	Passage experiments
Nothing abnormal noted up to 31 days  Sacrificed on 8th day  Sacrificed on 10th day	Nothing abnormal found  Slight congestion of both layers of tunica vaginalis	None found  None found	Brain, spleen, testes and adnexa of M 12 were ground up with 15 c.c. of normal saline 1 c.c. of this emulsion was injected into M.13, M 14 and M 15 M 13 had a temperature of 101° on 4th day, on 5th day showed a scrotal reaction It was sacrificed on 10th day Slight congestion of both layers of tunica were present, and scanty <i>Rickettsia</i> were demonstrated M 14 had slight inflammation of right side of scrotum and a temperature of 101° on 7th and 8th days No <i>Rickettsia</i> were found and no further passage carried out M 15 showed no reaction
No abnormality noted up to 21 days  Sacrificed on 6th day  Swelling subsided after 8th day Died on 23rd day of dysentery	Congestion of both layers of tunica Swelling and congestion of testes and scrotum Haemorrhages in polar fat Perisplenitis present	+	Testes and adnexa of 95 were emulsified in saline and two successful passage experiments were carried out, two out of three pigs in each case reacting as described in the text and showing pyrexia
Sacrificed on 6th day  Sacrificed on 6th day	Very slight congestion of tunica vaginalis and oedema of scrotum some perisplenitis  Very slight congestion of tunica vaginalis with oedema of scrotum	Scanty  Scanty	The testes and adnexa of 1A and 2A were emulsified in 20 saline and 5 c.c. of the emulsion injected into pig 14A. This pig showed a typical reaction and was sacrificed on the 8th day <i>Rickettsia</i> were found and subsequently six successful passage experiments were carried out



An occasional polymorph was seen in the walls of the smaller venules and arterioles. In some blood vessels the muscle cells of the media were swollen. The tunica itself showed swelling of the serosal cells with some hyaline degeneration. Capillary haemorrhages were present below both the visceral and parietal layers of the tunica vaginalis. The polar fat also showed haemorrhages in the severe reaction.

The liver and spleen were congested and there was some endothelial proliferation. We do not, however see true thrombus formation in these organs, although in some cases the smaller vessels were plugged with polymorphs. Vascular changes similar to those observed in the blood vessels of the testes and scrotal skin were seen in the heart vessels of one guinea pig.

We were unable to find vascular changes or focal necrosis in sections from the brains of infected animals.

We have not yet found the causal organism in smears of blood or in smears and sections of eschars removed from patients. We have made seven unsuccessful attempts to produce infection in guinea pigs by injection of emulsions of eschars in saline and have, therefore, been unable to repeat the experiments of TOROYO (1932) and ROBERTS (1939).

#### PRIMARY LESION OR ESCHAR

The most frequent site of the eschar which we assume to be the primary site of inoculation, was on the scrotum, the inguinal or umbilical region. To begin with, the lesion is similar to that of any tick bite. There is an inflammatory area of about  $\frac{1}{2}$  in. to  $\frac{3}{4}$  in. in diameter with a small central papule. This papule breaks down, leaving a necrotic black centre. By this time the eschar is about  $\frac{1}{4}$  in.  $\times$   $\frac{1}{4}$  in. in size. The lesion becomes smaller and the necrotic centre usually separates after about a week, leaving a clean but congested floor. The only difference between this lesion and the bites of clean ticks which we have observed is the tissue necrosis.

As has been said, we do not think that the presence or absence of an eschar is a criterion, as used by ROBERTS (1939) to separate the typhus-like diseases in Kenya into lesion and non-lesion types. The development of necrosis of the tissue may be due to factors other than the inoculation of the infecting organism. The site of the eschar suggests that friction or chafing may in part be responsible for establishing the lesion. Furthermore, secondary infection either from the mouth parts of the vector or from scratching may play a part. LAWTHWAITE and SAYOOR (1936) have discussed in detail how an eschar may or may not be found in *tritusugamushi* disease, and concluded that one and the same virus may cause gradations of dermal lesions that vary greatly in extent. We believe that this also applies to the tick-bite rickettsial disease under consideration.

On section of a fully formed eschar, the most marked changes, as in the case of guineapig lesions, are vascular ones. There is a central necrotic area which is sharply defined from the surrounding tissue. Below the fibrinous material of the necrotic area the epidermis is completely destroyed and is thinned out at the edges. The corium is oedematous and generally infiltrated with histiocytes and round cells. In several areas there are accumulations of round cells, and occasionally small areas of capillary haemorrhages are present. The vessels show complete necrosis in the places where they run into the necrotic centre of the lesion. In other places the vessels in the corium show endothelial proliferation, thrombus formation and medial infiltration similar to that seen in the vessels of infected guineapigs. Occasional vessels show plugging with polymorphs and round cells, giving a picture similar to that described in the vessels of the liver of one of the infected guineapigs.

In the sections of eschars we were unable to demonstrate rickettsiae in the endothelium or media of the diseased vessels. Sections of lymph glands showed a generalized lymphoid hyperplasia. The cells lining the lymph sinuses were swollen, and in some cases the sinuses were packed with endothelioid cells. No changes were found in the blood vessels of lymph glands and no rickettsiae were demonstrated in them. Unfortunately no biopsy specimens of skin have so far been available for study.

#### THE LEUCOCYTE COUNT

Total and differential leucocyte counts were done on thirty infected patients in Mombasa between May and August, 1944, by Major E J LOWBURY. A leucocytosis of 10,000 or over was found in 22 per cent., and a leucopenia of 5,000 or under occurred in 5 per cent of cases. The average neutrophil count was 50 per cent with 75 per cent or over in six cases.

#### WEIL-FELIX REACTION

Results of the Weil-Felix agglutination tests, also carried out by LOWBURY are given in Table V. The method recommended by FELIX (1933, 1944) was followed. Double dilutions of serum were made and set up in Kahn tubes with concentrated suspensions of *Proteus OXK*, *OX2* and *OX19*. The suspensions were obtained from the R A M C Laboratory, Kasauli, India. After incubation for 2 hours at 37° C the tubes were kept at room temperature for 18 hours and the results then read. By this method it is claimed that a labile antibody which would be destroyed at 56° C is allowed to act. All titres were read as "total" and "standard".

A series of forty cases was bled on the 5th, 10th and 15th days after the onset of symptoms. A fourth bleeding was to be taken on the 60th day but proved to be impracticable.

TABLE V  
WELL-FELIX REACTIONS.

Case name and number	Day of illness	Titre of total agglutination with			Titre of standard agglutination with		
		Protein XI <sup>9</sup>	Protein XII	Protein XK	Protein XI <sup>9</sup>	Protein XII	Protein XK
YA 1	0				640		40
	10				2,560		40
	15	2,560	0	160	10,240	40	1,280
DO 2	0						40
	10	0	0	80	0	0	160
	15	0	0	160	40	40	320
SA 3	5				80		40
	10				80		80
	15	0	0	80	80	40	640
SL 4	5	0	0	0	0	40	80
	10	0	0	80	0	40	160
	15	0	0	40	40	0	160
	20	0	0	0	40	0	40
BR 5	5	0	0	40	40	0	80
	10	0	0	40	0	0	160
	15	0	0	40	80	0	320
GA 6	5	0	0	0	0	40	40
	10	0	0	40	0	40	80
	15	0	0	40	0	0	80
HO 7	5	0	0	0	40	80	160
	10	0	80	0	80	160	160
	15	0	0	0	40	40	160
SM 8	5	0	0	40	0	0	80
	10	0	0	40	80	0	320
	15	0	0	80	80	0	320
CR 9	5	0	0	0	0	0	80
	10	0	0	0	0	0	160
	15	0	0	0	0	0	160
FM 10	5	0	0	0	0	0	40
	10	0	0	0	40	0	80
	15	0	0	80	80	40	160
LE 11	5	0	0	0	0	0	80
	10	0	0	0	0	0	80
	15	0	0	0	0	0	160
TH 12	5	0	0	0	0	0	80
	10	0	0	0	0	0	160
	15	0	0	80	0	40	320
WML 13	5	0	0	0	0	0	160
	10	0	0	40	0	0	160
	15	0	0	80	0	0	160

TABLE V—continued

Case name and number	Day of illness	Titre of total agglutination with			Titre of standard agglutination with		
		<i>Proteus X19</i>	<i>Proteus X2</i>	<i>Proteus XK</i>	<i>Proteus X19</i>	<i>Proteus X2</i>	<i>Proteus XK</i>
RU 14	5	0	0	0	0	40	80
	10	0	0	0	0	40	80
	15	0	0	40	0	0	320
BR 15	5	0	0	0	0	0	0
	10	0	0	0	0	0	80
TR 16	5	0	0	0	0	0	40
	10	0	0	0	0	40	40
	15	0	0	0	0	40	160
CP 17	5	0	0	0	0	40	80
	10	0	0	0	40	0	160
	15	0	0	0	40	40	320
CO 18	5	0	0	0	80	40	160
	10	0	0	0	80	0	160
	15	0	0	0	80	80	320
BG 19	5	0	0	40	160	80	320
	10	0	0	0	0	0	80
	15	0	0	0	80	40	80
BA 20	5	0	0	80	80	80	320
	10	0	0	40	80	40	320
	15	80	40	80	320	160	640
CH 21	5	0	0	0	40	0	80
	10	0	0	0	0	0	80
	15	0	0	0	40	40	320
RA 22	5	0	0	0	0	0	40
	15	0	0	40	0	0	320
MI 23	5	0	0	80	80	40	640
	10	0	0	40	40	40	160
	15	0	0	40	40	0	320
JI 24	5	0	0	0	40	40	80
	10	0	0	0	40	80	80
	15	0	0	80	80	160	640
BI 25	5	0	0	0	40	40	80
	10	0	0	0	40	40	160
	15	0	0	40	40	80	160
PZ 26	5	0	0	0	0	40	80
	10	0	0	0	40	40	80
	15	0	0	40	80	160	160
BC 27	5	0	0	40	40	40	160
	10	0	0	40	40	40	160
	15	0	0	40	80	40	320

TABLE V—continued.

Case name and number	Day of illness	Titre of total agglutination with			Titre of standard agglutination with		
		Protein XI <sub>1</sub>	Protein XI <sub>2</sub>	Protein XK	Protein XI <sub>1</sub>	Protein XI <sub>2</sub>	Protein XK
BK. 28	5	0	0	20	0	0	320
	10	0	0	0	0	0	160
	15	0	0	0	0	0	80
BHL 29	5	0	0	0	40	0	80
	10	0	0	40	0	0	320
	15	0	0	40	40	0	640
FG 30	5	0	0	0	40	80	320
	10	0	0	40	40	40	320
	15	0	0	160	160	40	1,280
RB 31	5	0	0	40	40	80	320
	10	0	0	40	0	40	320
	15	0	0	40	80	160	1,280
GU 32	5	0	0	0	40	0	80
	10	0	0	0	80	80	320
	15	0	0	0	160	40	80
MS 33	5	0	0	40	40	40	320
	10	0	0	40	40	40	160
	15	0	0	40	100	100	640
GL 34	5	0	0	40	40	0	640
	10	0	0	40	40	0	320
	15	0	0	40	80	40	1280
35	5	0	0	40	40	0	80
	10	0	0	40	40	0	160
	15	0	0	40	80	0	320
36	5	0	0	0	40	80	160
	10	0	0	0	80	160	160
	15	0	0	0	40	40	160
36	5	0	0	0	0	40	80
	10	0	0	0	40	40	80
	15	0	0	0	40	0	160
37	5	0	0	0	0	0	80
	10	0	0	0	0	40	80
	15	0	0	0	0	40	160
39	5	0	0	0	40	40	80
	10	0	0	0	40	40	80
	15	0	0	40	80	80	160
40	5	0	0	0	40	40	80
	10	0	40	0	40	40	160
	15	0	0	0	40	80	160

With the exception of one case (Case 1 in Table V) later proved to be a case of murine typhus, none of a total of 133 sera examined showed titres with *Protein* OX19 or OX2 above the upper limit of normality as defined by Trax (1:8 to 1:16). Only one case (Case 70) showed agglutination with OAK remaining 1+5 sec. at a dilution level of 1:160 to 1:200. The high standard titres obtained with OAK at 1 and 2 min. were 1:1,600 in seven and 1:250 in two out of the 133 cases in the stable, and 1:450 in three of another group of 5 cases. Granular agglutination occurred in one of four either bottle of OAK suspension, and it seemed probable that the suspension which yielded high standard titres with our sera were high purity. Trax and other workers have reported the absence of agglutination in OAK suspension, and the production of non-particle agglutination. Unfortunately this early supply of OAK suspension was exhausted before it was tested with sera from normal and malarial patients. However, the standard titre of sera from either group not differing from the infected subjects were never higher than 1:320, and later tests with sera from patients with the disease and from normal and malarial persons gave results in the region of 1:50 to 1:80 in OAK suspensions.

Our experience is similar to that of Piotrows (1936) working with Reda Mountain spotted fever in that the Weil-Felix agglutination reaction is not reliable diagnostically in mild and a sporadic natural infection, such as we have been dealing with in East Africa. In South Africa Gray (1938) found a high titer for fever cases in both OX2 and OX19 titres, but not with OAK.

Our serological findings are confirmed in a recent visit to Dr J. H. S. Gray of the South African Institute of Medical Research, and to Major A. N. Rooyen, R.A.M.C. of the Central Pathology Laboratory, Cairo. Apart from the one case of murine typhus already mentioned, agglutination tests with murine and epidemic typhus antigen suspensions were negative, and complement fixation tests carried out by Dr Gray were also negative.

### CONCLUSION

We believe that the outbreak of disease with which we were dealing at the RAF and prisoner of war camp in the Port Bentz area is identical with cases of similar symptomatology which occur sporadically throughout East Africa and adjacent territories. The disease is similar to, if not identical with the disease diagnosed as 'typhus' and recorded by many civilian doctors in East Africa. It has been shown by our experiments that two of the possible vectors of this disease are *R. sinensis*\* and *H. leachi*.

We cannot as yet make a dogmatic statement about the uniformity of the disease throughout East Africa. From a study of the literature and from unpublished records we believe that all cases of 'typhus' which have occurred in East Africa are essentially similar to those described by Watson

\* *R. sinensis* was incriminated as a vector of a similar disease in Eritrea (MASON & BAIN 1941).

(1945) and studied by us. There may be some variations in the strains of tick-borne *Rickettsia* in East Africa. Thus our animals inoculated with macerated *H. leachi* reacted more severely than those infected with *R. sinuatus*. Similarly guinea-pigs injected with blood from patients whom it was suspected had been bitten by *H. leachi* reacted more severely than those who had probably been infected by *R. sinuatus*. Our results to date are incomplete but there is a certain amount of evidence that in this tick-borne disease in East Africa there may be strains of *Rickettsia* of different virulence according to the tick vector responsible for the transmission of infection. At the same time we have found no difference in the morphology of the parasite, the pattern of cell infection or the pathological changes in guinea-pig tissues in

(a) animals infected with macerated ticks collected from Nairobi, and from Mombasa

(b) animals inoculated with blood from patients infected at the coast (Mombasa area) or in the highlands of Kenya (Nairobi)

It should be remembered that rickettsial diseases of the same group may show variations in their clinical pictures and that within any one group of *Rickettsia* there may be strains of varying virulence. This variation in the strain, perhaps determined by the tick vector may explain certain variations in the clinical picture of the same disease (e.g. CLEGG 1932; WALSH 1945).

At the same time it seems that there has been some confusion in East Africa with regard to the diagnosis and classification of rickettsial diseases. Murine typhus is known to be present in East Africa, as evidenced by the case diagnosed at Mombasa (Table I Case 1) and its presence is suggested by the experimental work of ROBERTS (1939). This latter investigator obtained a strain of *Rickettsia* and produced reactions in guinea-pigs by injection of emulsion of (a) fleas from rats, and (b) the organs of these rats. The rats in question were trapped at a brewery in Nairobi where four men had had typhus-like disease. ROBERTS stated that the available evidence pointed to the flea *Xenopsylla cheopis* as a probable vector of typhus in Kenya. He defined two forms of typhus in Kenya—"sanguineus-borne" and flea-borne. According to ROBERTS both types of typhus are much alike in their clinical features, but are distinguishable by the absence of an initial lesion and by their epidemiology. (It has already been stated that in certain of the cases, 19 per cent. of the tick-borne rickettsial disease under study no primary lesion was found.) According to ROBERTS the postmortem findings in guinea-pigs infected with the murine strain differ from those of the tick type mainly in showing less ascites and slight enlargement of the spleen. The rickettsiae demonstrated by ROBERTS in his case of "sanguineus-borne" typhus, which he kindly allowed us to examine, are in no respect similar to those found by us. The cells infected are serosal in type and are packed with rickettsiae. Our experiments using macerated fed

In our experiments ascites was only present in animals as a result of secondary bacterial contamination.

and unfed *Rhipicephalus sanguineus* (adult, nymphs and larvae) and eggs of *R. sanguineus* for inoculation into guineapigs are incomplete. We have produced pyrexia in one guineapig (Chart III, p 312) by injecting fed adult *R. sanguineus*, but we were unable to produce any reactions in guineapigs by passage from this animal nor in this case did we demonstrate rickettsiae. We have therefore, been unable to confirm ROBERTS' observation that *R. sanguineus* is a vector of tick typhus in Kenya.

ROBERTS laid emphasis on the contraction of the disease from ticks in tick-infested houses. We believe, as is evidenced by (a) the epidemiology of the disease, (b) the isolation of *Rickettsia* from *R. simus*, and (c) the results of the preventive measures at the prisoner of war camp, that the common source of infection is from ticks on long blades of grass. In our experience in the cases which are said to have contracted the disease in houses, a tick infested dog is the link to the original source of infection which is ticks on grass in the surrounding country.

The association of rainfall (ANDERSON, 1925; TONKING, 1932 and Fig 9b, p 303), and the incidence of the disease is of interest in this respect. During the rains the grass grows long and increases the chances of passers by being bitten by ticks.

For the prevention of the disease, the most important factors would seem to be (a) the cutting burning and keeping short of all grass near dwellings, (b) the avoidance of walking through long grass without protection from the attacks of ticks, (c) the avoidance of handling ticks when de-ticking dogs.

Normally sporadic cases of this tick borne rickettsial disease are found among Europeans in certain areas in East Africa and adjacent territories (Fig 1, p 297). When, however, a sufficient concentration of susceptible people is restricted to an infested area an epidemic such as was found in the Changamwe area (Fig 3, p 301) may occur.

We have no evidence as to whether or not there is an animal reservoir of the disease, and, if so, what it is. But if the disease like other tick-borne rickettsial diseases, is transmitted from adult ticks to their progeny the reservoir may be the tick itself.

Although we have not had an opportunity to complete our cross-immunity experiments,\* and have as yet had no success with tissue culture experiments,† there is sufficient evidence to classify this rickettsial disease as one of the Spotted Fever Group (PINKERTON, 1943). It differs from (a) the Typhus Group—both epidemic and murine and (b) the Tsutsugamashi Group and scrub typhus in the following ways—

- (1) the morphology of the parasite
- (2) the pattern of cell infection, with intranuclear forms,

\* These will be published at a later date

† Dr G L TIMMS and G W A DICK have so far had no success with the method described by ZINSSER, FITZPATRICK and WEI (1939)



- (3) the types of cells infected,
- (4) the histopathology of the disease in guinea-pigs,
- (5) the Weil-Felix reaction,
- (6) the low mortality in infected guinea-pigs,
- (7) the failure to produce infection in Egyptian gerbils (*Gerbillus gerbillus*),
- (8) the clinical picture in man,
- (9) the epidemiology of the disease,
- (10) the vector

If, as according to the evidence of American workers (PINKERTON 1943) the tick-borne rickettsial diseases do not belong to the Typhus Group of fevers, then the name tick typhus is an unsatisfactory one to describe this disease.

East African spotted fever or East African tick bite fever would then be more suitable. The name "tick typhus" is, however commonly used in East Africa in describing this disease and is in accordance with MEGAW's (1921) classification of the fevers of the Typhus Group. The use of the name tick typhus for this disease will probably lead to less confusion, and a better differentiation of the other types of rickettsial diseases in man in East Africa and adjacent territories.

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## TYPHUS IN NORTHERN NIGERIA I EPIDEMIOLOGICAL STUDIES

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During the period from 4th June, 1945, to 15th September, 1945, 126 cases of clinical typhus, of which thirty-two (25 per cent) died, were discovered in Jos native town, Northern Nigeria. Ninety-two of these cases developed Weil-Felix agglutination titres above 1 100. As far as is known, this is the first outbreak of epidemic typhus to be reported from British West Africa.

### EPIDEMIOLOGY

Jos town is divided into three distinct areas (a) the native town, with a population of approximately 20,000, chiefly Moslem Hausas, (b) the town-ship, with a population of nearly 5,000, made up of non-indigenous clerks and artisans of Southern Nigerian origin, and (c) the European reservation. Jos native market, the largest in the Plateau Province, attracts traders in large numbers from all parts of Northern Nigeria. These traders lodge in Jos native town for a few days or weeks, there is, therefore, a very considerable floating

\* We wish to thank Dr J W P HARKNESS, Director of Medical Services, Nigeria, for permission to publish this paper. Dr B G T ELMES, Assistant Director of Laboratory Services, Nigeria, Brigadier G M FINDLAY, Consulting Physician, West African Command, Accra, and Major GORDON, Officer Commanding Field Hygiene Unit, for their assistance and advice. We wish, also, to thank Dr HOWARD, Medical Officer, Jos African Hospital, for information regarding the cases occurring before our arrival. Our thanks are due to all members of the Health Staff for their untiring efforts, especially during the early weeks of the campaign, when shortage of staff necessitated long hours of work. Our particular thanks are owed to Mr S F BUNNING for his enthusiastic co-operation in organizing the campaign and obtaining for us much of the information published in this paper.

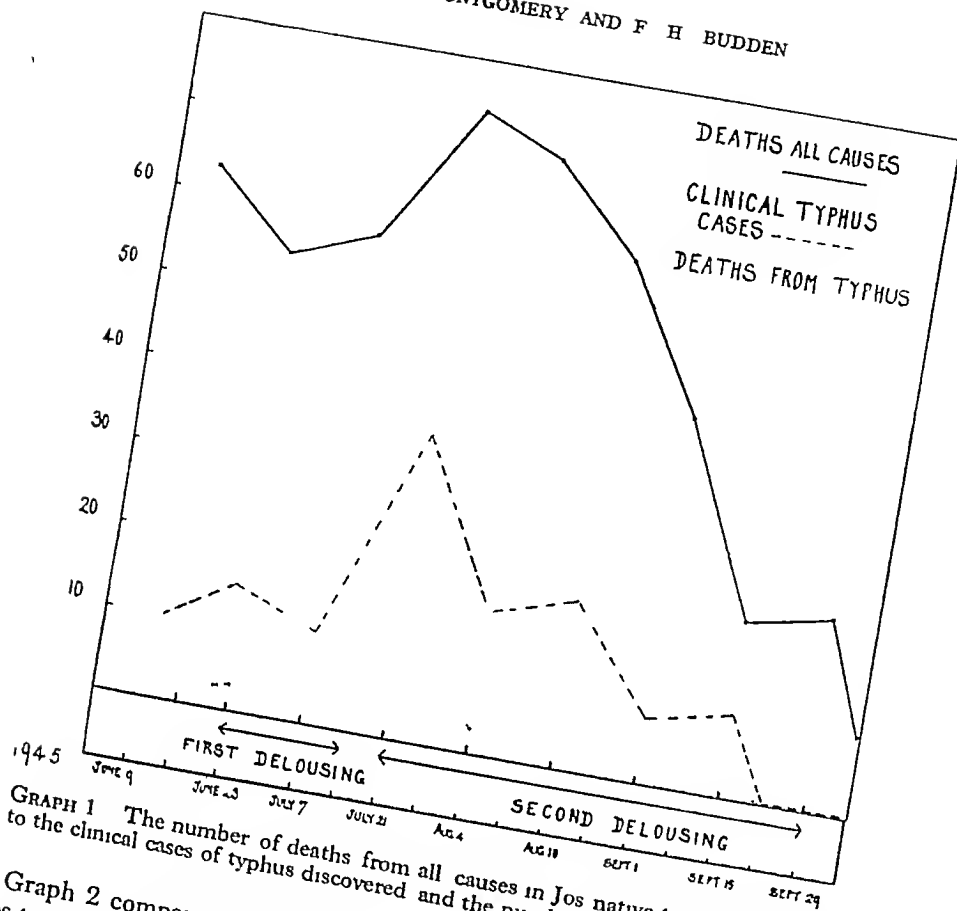
population, estimated at between 7 000 and 8,000 and included in the total of 20,000 for Jos native town.

Jos native town is divided into four approximately equal quarters. Each quarter is divided by roads into blocks and each block contains on an average ten compounds. A compound may house several families in a rabbit warren of dark, ill ventilated, overcrowded rooms of mud and thatch, with little, if any space between one building and the next. About half of the population in the native town do not possess a complete change of clothing. Washing of garments and personal ablutions are perfunctory. It was found that in the native town the population was almost wholly infested with *Pediculus humanus corporis* while head and crab lice were frequently found. In some cases the infestation was so heavy that it was impossible to find an inch of seam not inhabited by lice. Bed bugs were abundant on bedding and, occasionally on clothing.

### COURSE OF THE OUTBREAK.

During April, 1945 three patients admitted to Jos African Hospital were suspected of suffering from typhus. In two of these the diagnosis was confirmed by agglutination against *Proteus X19* at titres of 1 200 and 1 800 respectively in the third case no agglutination occurred. All these patients died. Although neither of us was stationed in Jos at the time, we understand that they presented a similar clinical picture to that of the severe cases occurring later. Endemic murine typhus is known to occur in British West Africa (FINDLAY *et al.*, 1943) and at that time no great importance was attached to the finding. As no registration of deaths was in force in the native town when we arrived it was thought that the opening of a new cemetery which took place at the end of May could be utilized to ascertain the crude death-rate. A caretaker was appointed to the new cemetery in order to make an accurate count of the number of burials each day. The first figures were obtained for the week ending 2nd June, 1945, during which twenty five burials were made from the native town. This corresponds to a crude death-rate of sixty five per thousand per annum, a figure greatly in excess of the normal for other towns in Nigeria. On enquiry it was found that prayers had already been offered in the mosque for the alleviation of a sickness in the town. The health staff were instructed to search for cases of sickness and on the 4th June cases were reported from one compound. The compound was visited and twelve sick persons were discovered. Further one person had recovered after a 16 days illness, and another had died 2 days previously after an illness lasting 12 days. Sera were taken from the patients and eight of these were returned positive to *X19* agglutination up to a maximum titre of 1 6400 in one case. Thirty-eight burials were recorded the next week.

Graph 1 shows, fortnight by fortnight, (a) deaths in Jos native town from all causes (b) the number of clinical cases discovered and (c) the deaths due to typhus amongst these discovered cases.

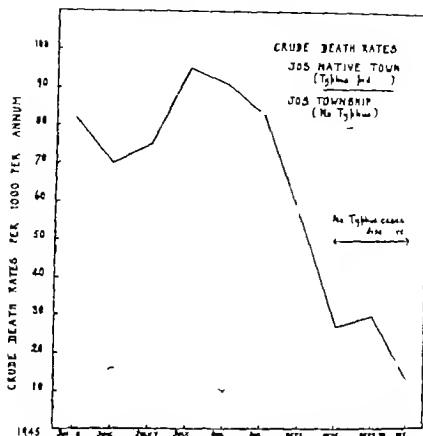


GRAPH 1 The number of deaths from all causes in Jos native town in relation to the clinical cases of typhus discovered and the number of deaths from typhus

Graph 2 compares the crude death-rate in Jos native town with the rate in Jos township, where no typhus occurred

Table I compares the incidence of discovered typhus cases with the crude death-rate in various parts of Jos, and also at Bukuru, a town 9 miles from Jos and the centre of the tin-mining area (Table I, p 331)

No doubt the ratio of discovered to undiscovered cases increased as our control measures became more effective. Allowing for this, it appears that the typhus very closely followed the incidence of discovered typhus (Graph 1). The discovered cases and total deaths continued to rise until a peak was reached in the middle of July. Thereafter, both fell until the middle of September. Since then no further cases of typhus have been discovered, and the deaths appear to have reached a base level averaging fourteen per week, which corresponds to a crude death-rate of 27 per thousand per annum.



GRAPH 2. The crude death rates from all causes in Jos native town, where typhus occurred, and in Jos Township where typhus did not occur

Unfortunately there are no previous figures available to show what is the normal death-rate for Jos. The rate in Jos township did not show any great variation from an average of fourteen per thousand per annum (Graph 2). This serves as a control of an African population exposed to the same climatic conditions but living under vastly more hygienic conditions. The lower incidence of typhus in Dodo quarter and Bukuru was associated with a lower crude death rate during the period of the epidemic (Table I.)

The crude death rate in Iano, a typical Northern Nigerian town, which includes areas similar to Jos native town and Jos township, has varied between twenty three and twenty five per 1000 per annum during the past 3 years. It seems probable that the death-rates in Jos have been "normal" since mid-September. During the 18-week period, 26.45 to 15.945, total deaths from all causes in Jos numbered 493, giving a weekly average of 31.1. During the

next 15 weeks, 15 9 45 to 29 12 45, when no typhus was discovered, total deaths from all causes were 164, giving a weekly average of 10 9. Amongst the sick examined, no other fatal disease was found to be abnormally prevalent. It is therefore probable that the excessive deaths in Jos native town during the period of the typhus epidemic were, in fact, due to typhus.

TABLE I

Area	Population	Number of clinical cases of typhus discovered	Incidence of typhus per 1,000 of the population	Crude death-rate from 27th May to 15th September
*Jos Native Town, Dodo Quarter	3 800	5	1 32	68 per thousand per annum
Jos Native Town, excluding Dodo Area	16 200	121	6 3	101
Jos Township	5 000	Nil	Nil	13
Bukuru	10 456	8	0 76	25

\* Dodo Quarter is the most modern part of Jos Native Town. Housing is better and it is not nearly so congested as the three other quarters, it is chiefly inhabited by Southerners with higher incomes.

#### DISTRIBUTION OF CASES

It is of interest that the 126 clinical cases discovered in Jos were admitted from fifty-eight compounds (there are 1,600 compounds in Jos). Moreover, out of a total of 240 deaths from unknown causes during the period 23rd June to 1st September, sixty-nine died in these fifty-eight compounds, an incidence of 1 19 deaths per compound in those compounds known to have been infected with typhus as compared with 0 17 deaths per compound for the rest of the native town. These figures suggest that case-to-case infection in compounds is usual.

*Climatic Conditions in Jos* Jos is situated on the central Nigerian Plateau at a height of 4,134 feet. The climate is considerably cooler, especially at night, than in Nigeria generally. There is no indication from meteorological returns for the past 3 years that the temperatures, rainfall or humidity have shown any variation of importance from year to year.

#### SUBSIDIARY OUTBREAKS

Between the 7th and 21st July eight cases of clinical typhus were discovered in Bukuru, an important tin mining centre 9 miles from Jos, and

connected with it by road and rail. Two of these cases were confirmed by Weil-Felix agglutination tests. All contacts were thoroughly disinfested and no secondary cases occurred. The death-rate in Bukuru did not vary from what is considered to be a normal rate, twenty five per 1 000 per annum during the period and it is probable that the infection was brought from Jos and did not gain any hold in Bukuru.

On 16th August in response to a report of undiagnosed deaths, one of us visited Vom, a village of about 1,000 inhabitants, 17 miles by road from Jos. Two cases of clinical typhus were discovered both were heavily louse-infested. A death, following a 7-days illness associated with "loss of sense," had occurred in one of the two compounds 10 days previously. Only one serum was collected before death, and this gave titres below 1:100 for the Weil-Felix reaction. The following day the inhabitants' clothing and bedding were deloused in both compounds. All the villagers were dusted with DDT powder. As the day was a public holiday and workers were at home, it is unlikely that more than a very small proportion of the population missed treatment. One further clinical case was admitted to Vom Mission Hospital within the incubation period, but no other cases have been reported.

### EPIDEMIOLOGICAL CONTROL.

Control of the epidemic was obtained by isolation of patients and the use of DDT dusting powder.

*DDT Dusting Powder.* Three types of DDT dusting powder were used. A small quantity (182 lb.) of Neocid, 5 per cent. DDT powder was all that was available during the first weeks. Later the Army and R.A.F. kindly supplied a quantity of crude DDT powder. Basalt clay which is a fine volcanic ash, produced locally by the Kaduna Syndicate, was found to make an excellent diluent for a 10 per cent. dusting powder. The mixing was a laborious process and had to be done by hand—it was carried out by Army personnel, who ground the two substances together with mason's trowels on flat boards. In this primitive way over 2½ tons of dusting powder were mixed. No ill-effects were observed in the persons engaged in the manufacture. Towards the end of August, mark III anti-louse powder (7.5 per cent. DDT) was made available in large quantities.

Three machines were used for dusting—

- (1) Hand operated dust guns.
- (2) Hand operated cyanogas pumps fitted with an extension hose and a wooden nozzle at the end. (See Figs. 1 & 2.)
- (3) Paint spray guns adapted by removing the fine jet and attached to petrol compressor pumps maintained at a pressure of from 60 to 80 lb. per square inch.





was repeated between the layers. For the bath clothing, approximately  $\frac{1}{2}$  oz. of DDT powder was used for each person. Women were employed to deal with the female part of the population in view of religious susceptibilities.

#### TECHNIQUE FOR DISINFESTING SPARE CLOTHING AND BEDDING. (See Fig. 2)

Compounds were thoroughly searched by the staff, and all clothing and bedding collected. Rags and old and torn sleeping mats were condemned and burnt. The remaining clothes were then taken one at a time laid flat, dusted on both sides, and between any layers. The next cloth was laid on top and similarly treated. When small bundle had been thus dealt with they were folded together and placed in galvanized iron dustbin fitted with lid, several dustbins being used in each compound. The dustbins were placed in the sun and left for 48 hours before opening. Use of dustbin had the following advantages —

1. They prevented removal, shaking and washing of garments, for 48 hours.

2. When left in the sun they became hot. Buxton (1945) states that heat accelerates the lethal action of DDT.

This method was highly efficient. No live lice were ever discovered on clothes treated in this way.

#### EARLY DISCOVERY ISOLATION AND DISINFESTATION OF CASES AND THEIR CONTACTS.

The combination of Moslem fatalism, with natural suspicion of the value of isolation in a disease which does not show a dramatic response to treatment, mitigated against early notification of cases of illness. In the beginning there was no notification at all and we were left to do the best we could by house-to-house searches. In order to enable the health authorities to discover and isolate cases the following steps were taken —

1. Compulsory registration of all deaths was introduced. The section refused permission to bury any body until burial permit had been issued by the Registrar. It was fortunate that in this Moslem community the method of burial is to use communal burial ground. There is no reason to believe that burials took place other than at the prescribed cemetery. This however did not give any indication of the cause of death, for autopsy was always refused by the Moslem community and in the majority of cases no medical certificate could be obtained. The compulsory registration, however did give us information as to where deaths were occurring in the town and which quarters were most affected. There was even then great difficulty in finding compounds where deaths occurred as streets were unnamed and neither blocks nor compounds were numbered. This difficulty was overcome by numbering blocks and houses ourselves.

2. Teams under the charge of native sanitary inspectors made systematic search from house to house. All cases of sickness discovered were examined by one of us, and if typhus was suspected the patient was deloused and removed to the isolation centre.

3. Disinfestation was carried out in all compounds from which suspected case was removed or in which a person died from any uncertified cause. All these compounds were subsequently visited daily for 2 weeks. Each day all inmates were seen and all cases of sickness examined by one of us. In this way secondary cases occurring amongst contacts in the same compound were discovered. During the early weeks of the campaign the inmates only were deloused but after the 21st July spare clothing and bedding were also treated.

From the 4th June until the 20th June owing to the limited supply of dusting powder it was only possible to disinfest persons in compounds in which deaths occurred from uncertified causes or from which a suspected case was removed.

By the 20th June, local production of dusting powder was sufficient to supply teams which went from compound to compound through the whole town disinfesting all persons. Compressor pumps were set up in the market to deal with lorry passenger traffic and people who were absent from compounds at the market. Approximately 600 people daily were treated in the market. At the same time disinfestation of all passengers leaving Jos by rail was commenced, using compressor pumps. At this time there was a general strike in the country and railway traffic was at a minimum. Only three trains a week left Jos, as compared with twenty-three in normal times. After the strike finished on 5th August, between 4,500 and 5,000 passengers left Jos weekly. This gives an indication of the population shift.

By the 14th July all the 1,600 compounds in Jos native town had been visited and the people disinfested. Altogether, during the period from 20th June to 14th July, 24,471 persons were treated with DDT powder. This includes general public in the market, train passengers and persons in compounds. The population of Jos native town is estimated at 20,000, and although there is a large shifting element we consider that only a small proportion of the people could have evaded treatment.

Despite this, the results of this first attack were distinctly disappointing. Two weeks after treatment compounds were examined for lice, 239 inmates were examined thoroughly and it was found that 63 per cent were still lousy. In order to assess the immediate results of treatment, these people were re-dusted. The infestation rate was reduced to 33 per cent in 24 hours and 19 per cent in 48 hours. No further change was found at 72 hours, and at the end of 1 week the infestation rate was 18 per cent. The following explanations are offered for the persisting infestations —

- (a) Washing of body and clothes before 48 hours had elapsed. Despite instructions to the contrary and their normal habits, it was obvious that clothes had been washed.
- (b) Changing into another garment which was already infested. Louse counts at this time on spare clothing showed 49 per cent infested.
- (c) Re-infestation from absentees and hatched nits after the powder had been shaken or washed off.

Owing to the poor results obtained with the previous method, it was decided to go through the town again, dusting all inmates, spare clothing, bedding, sleeping mats and mattresses. This was commenced on 14th July. The three-quarters of the town known as Zengi, Balarabi and Mantau were treated. In none of these compounds was a case of typhus discovered more than 14 days after treatment. The cases that subsequently occurred were probably incubating the disease and no secondary cases were seen.

#### PROTECTION OF STAFF WORKING ON THE EPIDEMIC

All staff received two injections of mixed epidemic and murine typhus vaccine at an interval of 1 week. The vaccine was prepared by the Connaught Laboratories, University of Toronto, and supplied by the Army authorities.

The African staff in full protective clothing were sprayed with DDT powder before coming on duty. Only one case occurred at an early stage among the staff: this was in an African female nurse on the 16th June, who had been nursing some of the first cases discovered, before prophylactic injections could be obtained. She recovered.

### DISCUSSION

From the experience gained in treating over 80,000 persons and 1,300 native compounds with DDT powder we would make the following comments and suggestions. These apply in particular to an unenlightened community which does not appreciate the value of prophylaxis and is therefore both unco-operative and evasive. We consider that the technique used for disinfecting people kills all living lice on body and clothing within 48 hours in at least 95 per cent. of a heavily infested population provided that re-infestation can be prevented. The sources of re-infestation are —

- (1) Changing into infested clothing
- (2) Contact with infested persons.
- (3) Hatching of nits.

Methods of preventing re-infestation from these sources are as follows —

(1) Treatment of all spare clothing at the same time as the clothing which is being worn. The method of placing dusted clothing in closed dustbins proved valuable. No lice survived this treatment.

(2) All possible contacts should be treated at the same time. Simultaneous treatment of persons in compounds, market and schools was carried out but this source of re-infestation is obviously difficult to eliminate.

(3) A repeat powdering after 7 to 10 days (about 7 days is the incubation period for nits). A very concentrated attack would be required to cover a large area in 1 week so as to be ready to follow up after 7 days. We found it impossible to do this in Jos.

In view of the obvious difficulties in eliminating sources of re-infestation, methods of preventing the removal of DDT from garments should be studied —

(1) Instructions not to shake or wash dusted clothes should be given. In Jos we found that these instructions were very frequently ignored.

(2) The possibility of solution impregnation of clothing should be investigated. According to Buxton (1945) clothing can be so impregnated that several washings are required to remove the DDT. A technique is required that would make it possible for a mobile team to collect all clothing, dip it in a DDT solution, dry it and return it to the owners. Such a technique would be of great value.

It may be stated that delousing of persons and the treatment of bedding and spare clothing in the town became extremely popular, so much so that householders used to enquire when their compounds could be done. The appearance of the delousing teams in the last few weeks of the campaign became the signal for householders to start bringing out bedding and spare clothing to be dusted. At a recent meeting between the local native council and the District Officer, the council expressed the hope that the 'fine powder' could be made available for purchase in the local shops. They further added that since the health authorities had powdered the town they had no lice and no bed bugs, moreover flies and mosquitoes had been reduced.

From the laboratory evidence brought forward by LINDLAY and FRISVOLD (1947) there is little doubt that the epidemic was one of louse-borne typhus.

### SUMMARY

An outbreak of typhus on the Bauchi Plateau of Nigeria is described. The control measures, in particular the use of DDT powder, are described. It is suggested that DDT impregnation methods might give the most satisfactory results in a native community.

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## TYPHUS IN NORTHERN NIGERIA II LABORATORY INVESTIGATIONS

BY

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The occurrence of an outbreak of typhus on the Bauchi Plateau in Northern Nigeria has been reported (in the previous paper) by MONTGOMERY and BUDDEN (1947). In the present paper the behaviour of the rickettsiae isolated during this epidemic is described, and evidence is brought forward that epidemic typhus is not confined to the Nigerian Plateau which, though within a few degrees of the Equator, might from its elevation be specially suitable for typhus to become epidemic.

The early history of rickettsial infections in West Africa has been reviewed by FINDLAY, REID and MAEGRAITH (1943) in describing the isolation of rickettsiae from the brains of rats and the blood of a European patient in Accra, Gold Coast.

There is now ample evidence to show that rickettsial infections are common in all the larger ports of West Africa, from Dakar in Senegal (DURIEUX, 1936) to Lagos in Nigeria (ELMES, 1943). These strains are apparently of murine type since they produce well-marked oedema in the scrota of male guinea-pigs, they have been isolated from the blood of patients as well as from the brains of rats, and they give rise in recovered patients to sera which agglutinate more strongly suspensions of murine than of louse-borne typhus rickettsiae.

Similar infections in man have also been observed in inland areas on the coastal plain of the Gold Coast some miles from Accra, and in the forest areas round Kumasi. Murine types of rickettsiae have been isolated from the brains of rats in both these regions. It is obvious that flea-borne typhus is widely distributed in West Africa and is not confined to the large ports.

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Unpublished observations strongly suggest that there is also a form of tick-borne typhus in West Africa. From the blood of a European officer stationed in Bauchi, Nigeria, in the autumn of 1943 rickettsiae were isolated in guinea-pigs while the patient serum gave a positive complement fixation test with South African tick typhus antigen but not with murine or epidemic typhus antigens. At the same time his serum failed to develop agglutinins against either murine or epidemic typhus rickettsiae.

Two similar cases also occurred at a few weeks interval in European sergeants stationed at Kuntumpe, Northern Ashanti Gold Coast. Here also no agglutinins developed for suspensions of either murine or epidemic rickettsiae.

The Bauchi Plateau outbreak is the first in West Africa which can be regarded as definitely epidemic in character. In the Belgian Congo however epidemics of rickettsial disease have recently occurred in Ruanda-Urundi, Coquilhatville, Léopoldville and Kasu. These epidemics, especially in the last 2 years, have given rise to a mortality as high as 6 per cent. (VAN HOOFF 1945) they are regarded as due to rickettsiae of murine type (WELFAN 1943; JADIN 1943a and b). In South Africa, GRAY *et al.* (1944) have reported an extensive outbreak of typhus which has continued in the Transkei since 1941. Here the evidence excludes a murine origin, transmission being almost certainly by lice.

#### ISOLATION OF RICKETTSIAE FROM THE JOS FEVERS.

Rickettsiae have been isolated both from lice collected in Jos and from the blood of patients suffering from acute infection.

Four batches, each of between thirty and forty lice, were employed. The first batch was taken by air from Jos to Lagos by Dr. CALVERT, Assistant Director of Health Service Nigeria, on 14th June, 1945. At Lagos the lice were washed, ground up in saline and inoculated intraperitoneally into four guinea-pigs. These guinea-pigs showed a rise of temperature to 104° F., or more on the 4th to 6th day after inoculation. Eighteen passages by intraperitoneal inoculation using emulsion of spleen and brain have now been made in guinea-pigs.

A few days later on 20th June, 1945 one of us (G. M. F.) flew to Jos with guinea-pigs, and further inoculations were then made with three batches of lice collected from the clothes of people who were either ill with a typhus-like disease or were living in the same compound as actual cases.

Each batch of from thirty to forty lice, after having been washed, was ground up in saline and inoculated intraperitoneally into guinea-pigs. The guinea-pigs from Batches 2 and 3 failed to react but those inoculated with Batch 4 showed a rise of temperature above 104° F. on the 5th, 6th and 7th days after inoculation. Passages were made using approximately 10 per cent. spleen and brain emulsion in saline. Twenty consecutive passages have now been made in guinea-pigs.

The blood of ten patients was inoculated intraperitoneally into guinea-pigs. As in most cases, the day of commencement of their illness was uncertain the blood was allowed to clot in the refrigerator. The serum was pipetted off and the clot ground up in saline. 2 c.c. of broken-up clot were inoculated intraperitoneally into three guinea-pigs. Two samples of blood from Beatrice O

who had almost certainly contracted the disease while nursing a patient, were inoculated on the 5th and 6th days of her illness intraperitoneally into three guineapigs. With the first sample of blood, two of the three guineapigs reacted 13 and 14 days after inoculation, while with the second sample of blood only one of the three guineapigs reacted after an incubation period of 13 days. In guineapigs the Beatrice strain has been passaged twenty times, using spleen and brain emulsion or blood intraperitoneally.

One patient, Shetu Zara, failed to produce any reaction in guineapigs which were subsequently not immune to a further inoculation of infected material while the patient herself at no time showed a positive Weil-Felix reaction. It is doubtful, therefore, whether this patient was suffering from typhus.

The ten strains isolated, with the approximate date of disease when the blood was taken, and the incubation period after inoculation of blood into guineapigs, are shown in Table I. The day of disease, except in the case of Beatrice O, must be regarded as approximate, many of the patients did not regard themselves as ill at all, while to the sick African and his friends time is not of great importance. The incubation period in guineapigs was calculated from the day of inoculation till the temperature rose to 104° F or above.

TABLE I  
STRAINS OF RICKETTSIAE ISOLATED FROM THE BLOOD OF HAUSA  
PATIENTS IN JOS

Name	Day of disease when blood was withdrawn	Incubation period in guineapigs in days
Beatrice (Strain 1)	5	13, 14
" ( " 2)	6	13
Eriyava	7	6, 6, 6
Hahima	7	7, 7
Dijé	5	9, 9
Hassana	5	6, 8, 8
El Haçj	3	10, 10, 12
Ibrahim	8	9
Yusufu	5	6, 6, 8
Ersabo	6	12

Ten strains were thus isolated from nine patients, twenty-one guineapigs out of thirty inoculated showing characteristic thermal reactions. These human strains have been passaged in guineapigs for from seven to twenty passages by intraperitoneal inoculation of either guineapig heart blood or emulsions of spleen and brain.





TABLE II  
RICKETTSIAL AGGLUTINATIONS IN PATIENTS FROM JOS

Number	Name	Epidemic.	Murine
1	Saidu	1 in 400	1 in 200
2	Sauda	1 in 3,200	1 in 200
3	Hajara	1 in 400	1 in 200
4	Kantari	1 in 800	1 in 200
5	Beatrice 0 (5th day)	Nil	Nil
	(10th " )	1 in 100	1 in 50
6	Musa Bukuru	1 in 400	1 in 200
7	Mama	1 in 400	1 in 100
8	Idibo	1 in 100	1 in 100
9	Ibrahim (5th day)	1 in 100	1 in 100
	(10th , )	1 in 1,600	1 in 200
10	Uba	1 in 250	1 in 100
11	Bukaru	1 in 100	1 in 50
12	Ibrahim Mohamud	1 in 100	Nil
13	Halima	1 in 200	1 in 800
14	Chin Chin	1 in 3,200	1 in 200
15	Alli	1 in 200	1 in 100
16	Yusufu	1 in 800	1 in 100
17	Serikm Kasua	1 in 200	Nil
18	Hakima	1 in 200	1 in 100
19	Hassan	1 in 400	1 in 200
20	Amma	1 in 200	1 in 50
21	Manu	1 in 100	Nil
22	Isa	1 in 200	1 in 100
23	Dodo (10th day)	1 in 200	Nil
	(25th " )	1 in 80	1 in 40
24	Garuba	1 in 800	1 in 200
25	Salau	1 in 50	1 in 100
26	Azimu	1 in 200	1 in 100
27	Shajbu	1 in 400	1 in 100
28	Gambo	1 in 200	1 in 100
29	Juman	1 in 800	1 in 1,000
30	Audu	1 in 1,600	1 in 80
31	Musa	1 in 100	1 in 800

RICKETTSIAL AGGLUTINATION IN WEST AFRICAN ARMY PERSONNEL

Number	Locality	Epidemic	Murine
1	Lagos, Nigeria	1 in 50	1 in 400
2	"	1 in 125	1 in 2,500
3	"	1 in 40	1 in 800
4	Accra, Gold Coast	1 in 20	1 in 200
5	"	1 in 10	1 in 100
6	Kumasi "	1 in 50	1 in 250
7	Teshi	1 in 40	1 in 400
8	Freetown, Sierra Leone	1 in 25	1 in 400
9	Bathurst, Gambia	1 in 40	1 in 800

gerbil cultures of the "Ette" strain of epidemic typhus, the "Ette" strain having been isolated from a native suffering from typhus in the Eastern Transvaal. The *R. prowazeki* var. *mooseri* antigen was similarly prepared from gerbil cultures of the "Rhodes" strain of murine typhus which was isolated from rats caught in the Paarl District of the Cape Province.

The results of complement fixation tests are shown in Table III.

TABLE III

Name.	Rickettsial complement fixation.		Agglutination of	
	Epidemic.	Murine	<i>Proxema</i> OX 19	<i>Proxema</i> OX 2
Mama	1 800	1 800	1 400	1 200
Idibo	1 1,000	1 280	1 400	1 100
Ibrahim	1 3,200	1 900	1 800	1 23
Uba	1 3,200	1 2,200	1 700	1 100

Although there is a certain amount of overlap showing the possession of common antigens, the complement fixation test, as in the case of the rickettsial agglutination test, favours the view that the strains should be classed as epidemic rather than murine. The results of complement fixation tests with the haduna strain are discussed below.

#### TRANSMISSION TO LABORATORY ANIMALS.

(1) Rabbits were inoculated intraperitoneally and intratracheally. Intraperitoneal inoculation sometimes produced a thermal reaction up to 104 F., but incubation periods varied from 6 to 14 days. Typical protocols are as follows —

*Rabbit 10* Inoculated intraperitoneally with 2 c.c. of 10 per cent. emulsion of guinea-pig spleen and brain from the seventh mousepass passage of Ibrahim strain. The temperature rose to 103.8° F. on the 6th day after inoculation: the rabbit died 4 days later. *Rabbit 12*, inoculated intraperitoneally with 2 c.c. of 10 per cent. emulsion of guinea-pig spleen and brain from the eighth guinea-pig passage of El Hadj strain: temperature rose to 104° F. on the 14th day and the rabbit died 8 days later.

On the other hand, other rabbits have either shown no thermal reaction or a transitory rise to 104° F. Blood removed on the 10th and 16th days has however given rise to reactions when inoculated intraperitoneally in guinea-pigs. Such rabbits have subsequently given positive Weil-Felix reactions and rickettsial agglutination, the titres reached with epidemic suspensions being considerably higher than with murine strains. A number of rabbits were inoculated intratracheally with suspensions of infected guinea-pig spleen and brain. The usual inoculum was 1 c.c. A rise of temperature above 104° F. invariably occurred on the 5th or 6th day after inoculation. On killing the rabbits on the 8th or 9th day pneumonic patches were present in the lungs and in diaphragm preparations rickettsiae could be detected after staining with Gram's stain.



<i>Leucocorys striatus</i> Linn.	Striped mouse.
<i>Lophocorys akrapoti</i> Temm.	Bush furred rat.
<i>Prionomys tullbergi</i> Thomas.	Bush rat.
<i>Clatighe spurrelli</i> Dollman.	Small dormouse.

The small dormouse often frequents houses and some of the specimens which proved susceptible in these experiments were caught making a nest in mattresses.

*Leucocorys striatus* is common in the savannah forest and grass areas around towns while *Lophocorys* has similar habitat. *Prionomys tullbergi* lives in secondary forest. For the above information, and for specimens of these rodents, we are greatly indebted to M. G. B. CAMERON, of the Forestry Department, Gold Coast. There are many other wild rodents in West Africa. Whether any of them are naturally infected with strains of *Rickettsia* pathogenic to man is a task for further investigation.

Although not native of the Gold Coast, the golden hamster *Cricetus auratus*, was found to harbour the Jos strain of *Rickettsia* in its brain and spleen up to at least 20 days after intraperitoneal injection with infected guinea-pig spleen and brain.

### TYPHUS FEVER IN KADUNA.

So far as the present epidemic was concerned, it appeared to be limited to the Bauchi Plateau. The following occurrence at Kaduna suggests, however, that this rickettsial infection was more widely distributed in Northern Nigeria. Incidentally it illustrates the difficulty in arriving at a correct diagnosis on clinical grounds alone.

#### CASE HISTORY

A nursing officer M. N. L., aged 23 years, who was serving in a military hospital at Kaduna, noted that on 27th July, 1945 she felt rather cold and shivery. She did not report sick. On the next day she felt off colour but in the afternoon washed her hair. During this procedure she noticed something hard adhering to her scalp just above the left ear. Eventually she separated off the lump and discovered that it was a small insect about  $\frac{1}{2}$  inch in length, oval and flattened in shape, brown in colour with diffuse mottling. The insect appeared to be differentiated into head, thorax and abdomen. It had 4 legs. Unfortunately she did not retain it. The same evening she sweated profusely.

On 29th July she felt a little better but was still far from well. She continued on duty however till the evening of 30th July when she felt very feverish, and noted rash. On admission to hospital she was found to be sweating profusely, temperature, 103 F, pulse, 124 respirations, 21 general condition, good, slight suffusion of both conjunctivae; tongue coated but moist, face flushed, with an obvious rash. The chest, cardio-vascular system and abdomen showed no abnormality. B.P. 110/75. On the scalp was a small ulcer about 5 mm. in diameter situated 2 inches above the left external auditory meatus. The ulcer was not tender, but the surrounding area was reddened and inflamed. On the 2nd day of hospitalization, blackish scab fell off leaving a clean raw surface which healed somewhat slowly. The cervical lymph nodes on the left side were enlarged but not tender. No other enlarged lymph nodes were present. The rash began on the upper part of the chest, neck and face and spread to the rest of the trunk and to the limbs, finally involving the palms of the hands and soles of the feet. At first the rash appeared as distinct maculo-papules, 2 to 4 mm. in diameter, the centre of each papule appeared as a slightly haemorrhagic spot; this central portion tended to disintegrate leaving a tiny shining pink scar. As the temperature fell the rash decreased, but was still discernible for nearly a week after the temperature was normal. There was no residual staining of the skin. The spleen was just palpable on the 3rd day after admission. There was some frontal headache for the first 3 days of illness but no pain in the eyes, apart from slight photophobia. The conjunctivae were congested for the 1st week. The temperature fell by lysis, the pulse rate corresponding with the temperature. The temperature was normal on the 10th day of admission to hospital, probably the 12th or 13th day of illness.

Repeated examinations for malarial parasites were negative. On 1st August, the 3rd day after admission, the total R B C count was 4,210,000 per c mm, Hb, 88 per cent, total leucocytes, 5,800 per c mm, polymorphonuclear leucocytes, 79 per cent, lymphocytes, 15.5 per cent, mononuclears, 5.5 per cent. On the 7th day after admission to hospital the total leucocytes were 7,800 per c mm, polymorphonuclear leucocytes, 70 per cent, lymphocytes, 25 per cent, mononuclears, 5.0 per cent. Blood cultures were bacteriologically sterile.

The patient made a rapid recovery. For the above information we are greatly indebted to Capt J DENFIELD, R A M C.

Weil-Felix *Proteus* reactions were carried out as follows —

2845	OXK	1 in 80	5845	OX2	1 in 80
	OX19	1 in 20	15845	OXK	1 in 80
	OX2	1 in 40		OX19	1 in 20
5845	OXK	1 in 80		OX2	1 in 320
	OX19	1 in 80			

Agglutination reactions with rickettsial suspensions were carried out with the same sera. The two earlier sera gave no agglutination but the last gave agglutination up to 1 in 100 with epidemic, 1 in 25 with murine, typhus.

As it was still thought that the case might be tick-borne infection, the last serum was sent to Major J GEAR, S A A M C, at the South African Institute for Medical Research, who carried out complement fixation tests with a number of different antigens as follows —

<i>Antigen</i>	<i>End Titre</i>	<i>Antigen</i>	<i>End Titre</i>
<i>Epidemic Strains</i>		<i>Murine Strains</i>	
"Ettie" S A epidemic	1 in 50	Rhodes S A murine	1 in 125
Breidl	1 in 50	Maritzburg	1 in 125
Madrid	1 in 50	<i>Tick-borne Strains</i>	
Middle East	1 in 50	Backhouse S A tick-borne	1 in 125
-		Amblyomma	1 in 125

The higher titres with the epidemic strains suggest that the infecting strain was due to an epidemic strain of *Rickettsia*. The patient had never been immunized against typhus.

On the 4th day after admission to hospital, probably the 6th or 7th day of disease, blood was injected intraperitoneally into guineapigs. These showed a febrile reaction on the 6th and 7th days after injection and on the second passage the thermal reaction was accompanied by a pronounced haemorrhagic involvement of the tunica vaginalis. The infection has been passaged fifteen times in guineapigs, the incubation period remaining very constant. All male guineapigs, from the second to seventh passages, showed a scrotal reaction, but this tended to decrease in intensity and in later passages was not seen. Guinea-pigs which had recovered a month previously from infection with the Jos strain were immune to the Kaduna strain, and *vice versa*.

There is unfortunately no evidence of how the patient became infected. Kaduna is 180 miles by road from Jos. The patient had not been out of Kaduna for 2 months prior to her illness and in addition Jos had been out of bounds for some weeks for the military. There were no other known cases of rickettsial infection either among patients in the military hospital or among the local civil population.

While the history of an insect bite and the presence of a primary eschar suggest a tick borne infection, the behaviour of the strain in guinea-pigs at first suggests a murine infection. On the other hand, the serological reactions are weighty evidence for an epidemic strain.

### DISCUSSION

Strains of rickettsiae which, from their isolation from the brains of rats, their behaviour in guinea-pigs, and the agglutinins they produce in the sera of recovered patients are undoubtedly of the murine type, are widely distributed in West Africa. In addition, there is evidence to suggest that there exists a form of tick borne typhus. So far however there is no evidence that either of these types occurs except in sporadic form.

The present outbreak on the Bauchi Plateau is the first recorded from West Africa where rickettsiae have been isolated from lice and is also the first recorded epidemic of any rickettsial infection.

The evidence that the rickettsiae involved were of the epidemic rather than of the murine type, apart from the isolation of rickettsiae from lice, is derived from the following findings —

(1) The type of reaction in guinea-pigs.

(2) The type of agglutinins found in the sera of patients and animals recovered from infection.

(3) The results of complement fixation tests.

(4) The symptoms and pathology of the disease in man.

**Reactions in Guinea-pigs.** At first the appearance of scrotal reactions in guinea-pigs suggested infection with a murine type of rickettsia. The non-progressive scrotal oedema, its disappearance often before the fall in temperature and its increasing rarity with continued passage in guinea-pigs, are all in contrast with the behaviour of true murine strains isolated in West Africa. In South Africa, Gear (1945) informs us that he has isolated numerous strains of epidemic rickettsiae which, when first passaged in guinea-pigs, produced an atypical scrotal oedema. Later this tendency to produce orchitis disappeared. It is possible that many true epidemic strains, when first isolated into guinea-pigs, have behaved in a similar manner or as in the case of the Nicolle Tunis strain, have continued to produce orchitic reactions in a small percentage of male guinea-pigs (Zemmer and Castaneda, 1933).

**Agglutination Reactions.** There were occasional sera which gave the same end point with suspensions of murine and epidemic rickettsiae and a very few

which agglutinated murine more highly than epidemic rickettsiae. The majority, however, gave higher titres with epidemic than with murine rickettsial suspensions. Agglutination with the sera of recovered rabbits and guinea-pigs also showed higher titres with epidemic than murine rickettsia.

*Complement Fixation* In the same way complement fixation was in four of six cases more pronounced with epidemic than with murine antigens. It would seem that epidemic and murine strains, while possessed of specific antigens, also contain common group antigens.

*Symptoms and Pathology* Although many of the patients were ambulant and far from ill, the majority showed broncho-pneumonic signs and mental torpor, often accompanied during convalescence with tremor and myocardial involvement. Of the two cases coming to postmortem, one showed widespread involvement of the central nervous system, the other of the heart muscle. Despite the fact that many mild cases were probably never brought to light, a mortality of nineteen out of ninety-three cases is very high for murine infection even among a poverty-stricken population.

Finally, the scarcity of rats in Jos renders it far from easy to see how they could have been responsible for so considerable an epidemic. The passage of the rickettsiae through *Xenopsylla cheopis* is not evidence that the rickettsiae are of murine type, for many observers have passaged epidemic rickettsiae through rat fleas. In comparison with lice, with which the Hausa swarmed, human fleas were rare, nor was there any evidence to incriminate dogs and their ticks.

The evidence is thus all in favour of the view that the outbreak of typhus in Northern Nigeria was due to rickettsiae of the epidemic type. This is in conformity with what has been found in South Africa, but in the Belgian Congo VAN HOOFF (1945) and others regard all outbreaks as due to rickettsiae of murine type. Serological investigations on the strains involved do not appear to have been carried out.

In West Africa, as in South Africa, it thus seems probable that there are three types of rickettsial infection—murine, louse-borne and tick-borne. The geographical distribution and the exact behaviour of these infections in countries close to the Equator, require far more intensive investigation than they have yet received. There is, however, evidence that louse-borne typhus is present in Northern Nigeria.

## CONCLUSIONS

During the Bauchi Plateau outbreak strains of rickettsiae were isolated from two of four batches of lice collected from infected compounds. Rickettsiae were also isolated from the blood of nine African patients in Jos and of one European at Kaduna, some 180 miles from Jos.

Strains passaged in guinea-pigs in their early stages gave rise to orchitic reactions in male guinea-pigs. In later passages this power was lost.



Fatal results in guinea-pigs were not uncommon. Rickettsiae were widely distributed in the blood, peritoneal and pleural exudates and urine of infected guinea-pigs.

Rickettsiae could be demonstrated in liver, spleen, bone marrow, kidney and lung by suitable staining methods.

All strains of rickettsiae from Northern Nigeria gave cross immunity in guinea-pigs; they also showed cross immunity with a murine strain of rickettsiae isolated from rat brains in Accra, Gold Coast.

Dogs, rabbits, white mice and wild West African rodents *Cricetomys gambianus*, *Leptomys striatus*, *Lophomys sikanius*, *Prasomys tallbergi* and *Clavia spurelli* were successfully infected. The golden hamster *Cricetus auratus* was also susceptible. Among monkeys infection was inapparent except in *Cercopithecus aethiops centralis*.

The rickettsiae were experimentally passaged through rat fleas *Xenopsylla cheopis* but not through dog ticks *Rhipicephalus sanguineus*.

Sera from patients and from experimentally infected rabbits and guinea-pigs showed agglutination in higher dilutions with epidemic than with murine rickettsial suspensions. Complement fixation tests with human sera from Jos and Kaduna showed greater reactions with epidemic than with murine antigens.

The need for further investigation into the rickettsial infections present in West Africa is stressed.

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Since this report was written two cases of house-borne typhus have been reported from Kano during the later months of 1945, to be followed in the first few months of 1946 by a small epidemic in Kano City.

## TYPHUS IN NORTHERN NIGERIA III CLINICAL STUDIES

BY

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AND

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In previous communications the epidemiology of the outbreak of typhus on the Bauchi Plateau in Nigeria in 1945 has been described (MONTGOMERY and BUDDEN, 1947, in this number, page 327), and evidence has been brought forward to show that the infection was caused by the rickettsiae of louse-borne typhus (FINDLAY and ELMES, 1947, in this number, page 339)

Since epidemic typhus in natives of West Africa has not been previously reported, a brief account is given of the main clinical findings in this epidemic, the data being based on fifty-three cases which were confirmed by Weil-Felix agglutinations at titres above 1/100. The remainder of the patients were admitted at a late stage of the disease when they were irrational and no reliable statements could be obtained.

*Clinical History* Patients were generally admitted between the 4th and 10th days of illness, and if still rational gave a history of sudden onset (they had been working the previous day) with frontal headache, pain behind the eyes, and fever. This was usually followed by pains in the limbs and back.

\* Our thanks are due to Dr J W P HARKNESS, Director of Medical Services, Nigeria, for permission to publish this paper, to Dr B G T ELMES, Assistant Director of Laboratory Services for details of the histological findings, and to Dr HOLLINS and Dr UNDERWOOD for examining cerebrospinal fluids.

Pain was often localized to the large joints (knee, ankles, shoulders, elbows and wrists)

*Symptoms.* In addition to the pains, they complained of dryness of the mouth and throat (except in the few patients who showed excessive salivation), and, in more severe cases, of soreness at these sites. Cough was a common symptom, usually commencing about the 3rd or 4th day of the illness. Bowel symptoms or vomiting were never complained of although on direct questioning it was found that constipation occasionally occurred during the first few days. Various nervous symptoms were complained of inco-ordination, deafness, etc.

*Course.* The temperature was usually maintained between 102° and 105° F until about the 9th day when it began to fall by lysis. The lysis was gradual and a normal temperature was reached on the 8th to 14th day (average 12th day). Prostration and mental depression appeared early but tended to increase whilst the fever persisted. Other signs of encephalitis appeared during the period of maximal fever but became more marked during lysis. In those patients who later recovered improvement was noted the day after temperature reached normal. The mental state improved rapidly although tremor inco-ordination and paresis often persisted or increased. These nervous signs began to decrease during the 1st week after lysis and thereafter convalescence was rapid.

*Signs.* Toxicæmia and prostration were marked during the early stages of fever but encephalitis developed rapidly and dominated the clinical picture. A characteristic facies was almost universal, bloated and expressionless with mouth ajar injected conjunctivæ and ptosis. Patients were often unable to swallow food and in severe cases dehydration and wasting led to a sunken facies and concave abdomen. Histories could generally only be elicited with difficulty. The patient was lethargic or stuporous and occasionally deaf. Moreover the speech was often inco-ordinated and its clarity further impaired by laryngitis. Patients with severe nervous manifestations found considerable difficulty in protruding the tongue. They would open their tremulous lips make movements from side to side with their tongues, and then with obvious difficulty bring a shaking hand to the mouth, overshoot the mark, and finally try to pull the tongue out with their fingers.

#### CLINICAL PICTURE IN DETAIL.

*Cardio-vascular System.* The fall in temperature was not accompanied by a corresponding fall in pulse. A high pulse-rate often persisted for some days. This was especially marked in fatal cases, but in one patient, who later recovered, a resting pulse of 132 was present 10 days after temperature returned to normal. Mixed beats were not uncommon. No dilation of the heart or abnormality of sounds could be demonstrated clinically. It was not possible to record blood pressures.

*Respiratory System.* Inflammatory changes in the upper respiratory mucosa were common. A deep red injection of fauces and mouth appeared

early and was usually accompanied by an irritant non-productive cough and a hoarseness of voice due to laryngitis. Respiration rates were commonly raised out of proportion to the temperature and without any physical signs in the chest. Inco-ordination of respiration occurred in some of the patients with severe nervous involvement. Bronchitis was a common complication towards the end of the fever, sputum was copious and watery, and moist crepitations were scattered over both lung fields. In one patient there were signs of consolidation confined to the left lower lobe. These appeared during lysis and resolved rapidly without administration of sulphonamides.

*Alimentary System* The lips were dry, cracked and covered with sordes. The tongue was characteristic, coated with a thick central road of fur. The colour was white, brown or, in the most severe cases, a dirty black. Usually the tongue and mouth were extremely dry on admission but salivation returned rapidly at the commencement of lysis and was often excessive at this stage. Ulceration of the buccal mucosa occurred in two patients and a frank parotitis complicated a third, this resolved without specific treatment. Owing to inco-ordination of deglutition and soreness of the mouth and throat it was difficult to maintain nutrition during the acute stage.

*Jaundice* occurred in five patients (9 per cent).

*Renal System* Albuminuria was present in every patient on admission, and casts were present in all but two patients. These signs cleared up spontaneously. *Inter*

*Spleen* This was enlarged in twenty-four patients (46 per cent). Although this rate is above normal, it is of no diagnostic value in a malarious region.

*Central Nervous System* Mental state. This varied considerably, but some mental impairment was present in all but four cases (8 per cent). In severe cases the depression progressed from apathy through lethargy and stupor to coma and death, but in the less severe cases did not progress beyond one of the earlier stages. The maximal degree of mental involvement reached was coma in nine cases (17 per cent), lethargy, seventeen cases (32 per cent), apathy, five cases (9 per cent).

Lethargy was the commonest finding on admission. The patient lay immobile and expressionless and appeared to take no interest in his surroundings. When a question was asked or an order given the reply or reaction, if any, was unsatisfactory. Persistent questioning in a loud deliberate voice was rewarded with a satisfactory answer, given in a slow expressionless voice. Memory and orientation were normal. Instructions (*e.g.*, putting out tongue), if repeated or demonstrated, were eventually carried out, but slowly and with seeming effort. The tongue, once protruded would be left out indefinitely until further instructions were given. In the stuporous patients the picture was similar but they were dis-orientated as to time and place. Instructions were not always understood. This stage merged into coma. None of the nine patients developing coma recovered.

Delirium was less common than stupor and was usually transient. The patients were sometimes violent and sometimes in a "low muttering state. The acute catatonia shown by one patient only lasted 2 days. The appearance was classical. The patient recovered.

*Involuntary Movements* Only 18 (34 per cent.) of the patients did not develop involuntary movements. The commonest was tremor in twenty-seven cases (51 per cent.) first noticed as a fine tremor of the protruded tongue. Later tremor appeared round the mouth and in the outstretched hands. Less commonly the legs were involved. In more than half the cases the tremor became coarse. Tremor became more marked on intention (e.g., pointing test) and was not accompanied by rigidity. Well marked choreiform movements were present in six cases (11 per cent.), they involved the same sites as the tremor. Athetosis occurred in two cases (4 per cent.) one died. Fibrillary tremor of arm and leg muscles was often noticed during recovery of severe cases.

*Inco-ordination* was demonstrated in all those patients (68 per cent.) who developed involuntary movements. It appeared at the same time and ran a parallel course, although it generally persisted rather longer than the involuntary movements. Tip of finger to nose pointing test was inaccurate sometimes grossly so, but was not made worse by closing the eyes. Rapid alternate pronation and supination of both forearms could not be performed (adiadokokinesis). If the patient was able to stand with feet together and eyes shut, swaying was obvious. Gait was unsteady and on a wide base during the early stages of convalescence. Position sense was, however normal, i.e. patients were able to tell whether toe or finger joints were flexed or extended.

Muscular power was not diminished. There was no alteration in tone. Nystagmus was only seen in two patients (it occurred to both sides in the horizontal plane).

*Reflexes* were abnormal in forty two cases (79 per cent.) They were usually diminished during the pyrexial stage but became brisk soon after temperature fell to normal. The abnormality was more marked in the arm jerks than in the legs (corresponding with the distribution of maximum tremor). The increased ankle jerks were often accompanied by clonus. It was not always possible to obtain a plantar reflex owing to the thickened soles of the feet. Abdominal and plantar reflexes were absent in coma. With these exceptions the superficial reflexes were normal. Rabinaki's sign was never encountered.

*Speech*. Slurred monotonous speech was the rule. In some of the more severe cases speech became explosive and transitory aphasia developed in two cases (4 per cent.). Transitory deafness developing early in the pyrexia, and, lasting on an average 7 days, occurred in four patients (8 per cent.). In the most severe cases it was complete.

*Facial Palsy*. Unilateral facial palsy occurred in five patients (9 per cent.). Its onset was delayed until after the pyrexia and it remained in evidence for about a week. It was only partial and was most marked around the mouth.

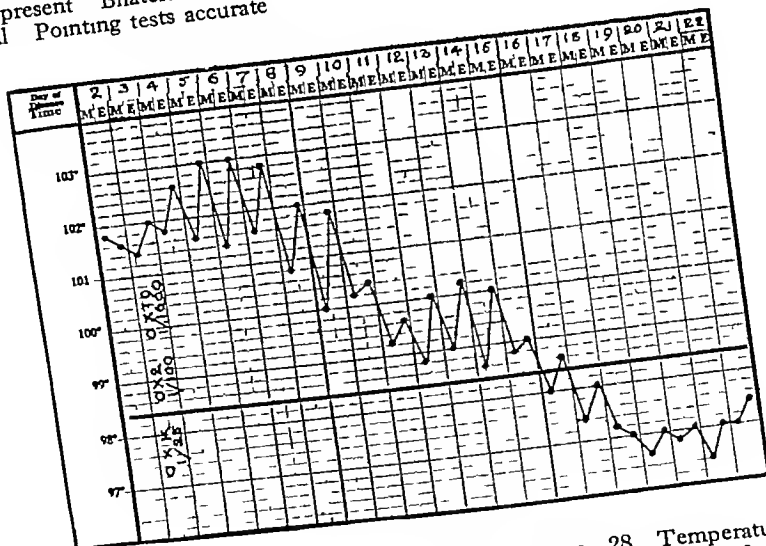
It was more obvious on involuntary than voluntary movements and was considered to be of an upper neurone type. In one patient, Musa, the lesion was bilateral and associated with bilateral fifth nerve and unilateral third nerve palsy on the right side

Persistent hiccoughs occurred in one patient the day after the temperature reached normal. He became comatose 3 days later, and died the following day

### CASE HISTORIES

**Dodo** Male, aged 16 years. A typical case. Admission, 18.8.45. He has been ill 2 days with fever but will not admit to any other symptoms.

**Clinical findings on admission —**  
 Temp, 101.8° Pulse, 120 Resps, 36 Drowsy Facies slightly puffy round the eyes  
 Conjunctivae injected but no jaundice Tongue, thick white fur but moist No rash  
 Spleen not enlarged Chest, n.a.d Heart, n.a.d CNS, n.a.d  
 19.8.45 Urine heavy precipitate of albumin and granular casts W.F. agglutination XK, 1 25, X2, 1 100, X19, 1 1,600  
 25.8.45 (9th day) Temp, 100° Pulse, 124 Resps, 30 Tongue slightly furred  
 Facies bloated, expressionless, with ptosis, and mouth slightly ajar Lethargy has developed since admission. He is slow at understanding but answers questions after an interval Speech slurred and jerky Very gross coarse tremor of protruded tongue, but no other involuntary movements Reflex jerks all very brisk in arms and legs Abdominal reflexes all present Bilateral ankle clonus Bilateral flexor plantar response Power and tone normal Pointing tests accurate



30.8.45 (14th day) Temp, 98.6° Pulse, 120 Resps, 28 Temperature has been falling by lysis Speech improving, no longer jerky Very coarse tremor of upper limbs, head and face has developed More marked on intention Pointing test (finger to nose) inaccurate, probably owing to intention tremor Rapid pronation and supination of fore-arms inco-ordinated Position sense accurate in finger and toe joints No Rombergism on standing with feet together, whether eyes open or closed Reflex jerks still brisk and ankle clonus still present

4.9.45 (19th day) Temp. 97.2° Pulse, 90 Resps., 20. Facies no longer bloated and more mobile. Very fine tremor around mouth and of upper limbs. The lower limbs also show fine tremor now. Reflex jerks—Upper limbs: R.J. normal L, brisk R; T.J., brisk both sides S.J. normal both sides. Lower limbs: K.J. and A.J. are both brisk. Ankle clonus present, but not as well marked as previously. Position sense accurate. Patient has started walking but is rather unsteady probably owing to debility for there are no other signs of loco-ordination.

6.9.45 (21st day): Tremor is now only present around mouth and is very fine. Reflex jerks are now all normal except ankle jerks, which remain brisk. Ankle clonus present. Walking steadily.

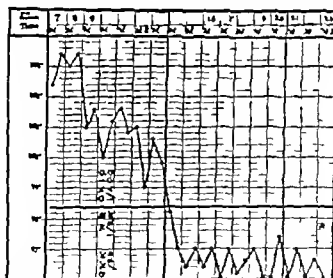
9.9.45 (24th day) Completely recovered. N.C.N.S. signs. Mentally normal. Discharged.

15.9.45 (30th day) Follow up examination. Says he is now back at his old work as carrier in the market. R. says he is as strong as before the illness and has no symptoms. C.N.S., n.a.d.

**MAIRIGA.** Female, aged about 38 years. A typical case of severe type. Admission, 9.7.45. She has been ill 7 days with fever intense frontal headache, pains in the lumbar region and pains in the limbs.

Clinical findings on admission —

Temp 102.6 Pulse, 140 Resps., 32. Marked prostration. Lethargic under stands questions, and answers in monotonous voice when they are repeated. Facies: slightly bloated, immovable, peevish. Conjunctivae injected but no jaundice. Tongue dry with central rod of thick brown fur. No rash. Spleen not enlarged. Chest, n.a.d. Heart, n.a.d. C.N.S. N. tremor power and tone normal. All reflex jerks brisk, especially biceps and supinator jerks. Response equal on both sides. Abdominal reflexes all present. Bilateral Babinski plantar response.



10.7.45 Urine heavy precipitate of albumin on boiling granular casts. W.F. admission AA, 1.25 X 1.25 X 1.25 X 1.25

12.7.45 Temp. 101.6°C. Pulse 132 Resps. 30 Mental condition deteriorating. Now stuporous. Patient does not answer questions, although she appears to have vagu

idea of what is being said and occasionally nods head in reply She put out her tongue very slowly after having been asked three times in a loud voice We had some difficulty in persuading her to put it back again Not taking food except little fluid by mouth

17 7 45 (15th day of illness) Temp, 96 6° Pulse, 96 Resps, 30 Temperature has been falling by lysis and reached normal yesterday Low muttering delirium Eyes and cheeks sunken. Fine tremor of upper limbs

19 7 45 Now semi-comatose Tremor of head and upper limbs very coarse

23 7 45 Deeply comatose No involuntary movements now

25 7 45 Died in coma

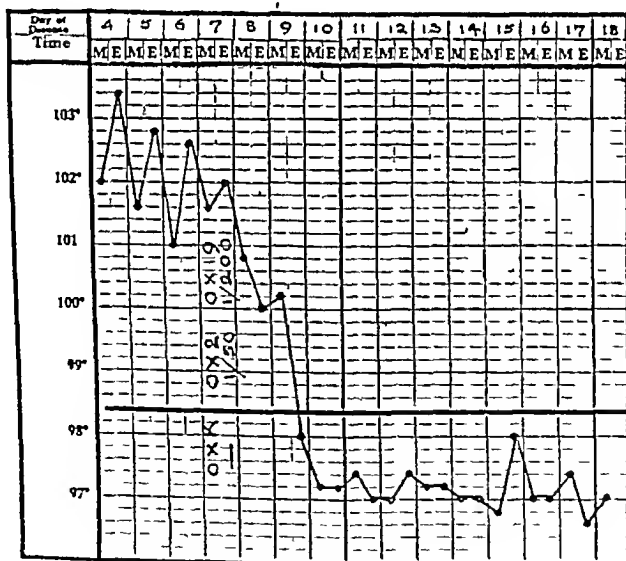
SHAIBU Male, aged about 47 years Typical mild case Admissions 30 8 45 Says that he has been ill 4 days with fever, frontal headache and pain, all over He has had a sore mouth for 2 days No cough

Clinical findings on admission —

Temp, 103° Pulse, 120 Resps, 30 Face expressionless Tongue dry, dirty white fur Lethargic, answers questions quite rationally, but is rather slow and sometimes appears to take no notice until question is repeated Spleen not enlarged CNS Reflexes, power and tone all normal No involuntary movements

31 8 45 (5th day) Urine, albumin No casts seen WF agglutination tests XK, -ve, X2, 1 50, X19, 1 200

4 9 45 (9th day) Little change in mental condition, but has had increasing deafness since admission Can now only hear a loud deliberate voice, otherwise no CNS lesion discovered Temperature had been falling by lysis, and reached normal on the evening of 4 9 45



6 9 45 (11th day of illness) Temperature has been subnormal for 2 days Tongue moist and only slight fur Now much drowsy Slight tremor of upper limbs and of protruded tongue Reflex jerks brisk in upper and lower limbs Abdominal reflexes all present Bilateral flexor plantar response Finger to nose pointing test overshoots the mark Rapid pronation and supination of forearms inco-ordinated Position sense in joints of fingers and toes accurate



12.9.45 (17th day of illness) No tremor now. Hearing normal. Mentally quite alert, and laughs at joke. Reflex jerks: Upper limbs—biceps, triceps and supinator jerks all very brisk on both sides. Lower limbs—response is normal on both sides. Abdominal reflexes normal. Plantar responses flexo. Pointing test now accurate and gait normal.

15.9.45 (20th day of illness) Reflex jerks still brisk in upper limbs, but no other abnormality discovered. For discharge tomorrow.

## SPECIAL INVESTIGATIONS

*Laboratory investigations* have been reported in a separate paper by FINDLAY and ELMER (1947 in this number page 339).

*Cerebrospinal Fluid.* Fourteen lumbar puncture fluids were collected from a consecutive series of patients during the period of temperature lysis. Two of the fluids showed xanthochromia, but in the rest the fluid was clear. In one patient, Musa, mentioned above, the cell count was 420 per c.mm. (mostly lymphocytes). In the remaining patients the count varied between 4 and 102 cells per c.mm. (average 33). Lymphocytes always predominated but a few polymorpha were also seen.

The total protein was usually raised and varied between 21 and 102 mg per cent. (average 69 mg per cent.). The protein level did not appear to be related to the cell count. Unfortunately owing to lack of laboratory facilities, no other investigations could be made.

*Differential White Cell Counts.* These were done on a series of four patients only. In one of them, Rakya, a high polymorph leucocytosis was present which was presumed to be due to an acutely inflamed septic guinea-worm ulcer complicating the picture. In two of the patients the total and differential counts were within normal limits and in the remaining patient there was a leucocytosis of 15,300 with 56 per cent. polymorpha and 41 per cent. lymphocytes.

## POSTMORTEM

This was refused in all but two cases.

We are indebted to Dr HOWARD Medical Officer Jos African Hospital, for the findings on the first case.

Gajere Gombe, pauper aged 16, admitted from Jos market on 13.4.45 complaining of cough, fever, general pains in limbs and body.

There was fever with restless delirium, meningism and head retraction, cutaneous facial spasms and stertorous apnoea of the upper limbs. There was cry every now and then. Tongue furred, abdomen retracted, spleen palpable. Knee jerks absent. C & F test and under no pressure. 50 lymphocytes per c.mm. No rash. Albuminuria. Temperature remittent between 101 and 103°F. Diagnosis of encephalitis. Coma and death on 18.4.45. Later Weil-Felix agglutination was reported as 1:800 against Proteus OX19.

At autopsy the brain was haemorrhagic and showed histologically foci of round cells, perivascular infiltration, haemorrhages and nerve cell degeneration.

2. Fort Lamy. This patient a pauper was admitted in coma. He was said to have been ill 5 days.

Serum collected on the day of admission showed Weil-Felix agglutinations of *XX*, 1 25, *X2*, 1 400, and *X19* nil. The patient died the same night.

*External appearances* Male subject about 30 years old, with bloated facies and slight icterus of conjunctivae. No rash seen.

#### *Macroscopical findings*

*Heart* Normal size and appearance, but petechial haemorrhages on intraventricular septum.

*Trachea* The mucosa was injected.

*Lungs* No free fluid in pleural cavities. The pleural surfaces at the base of the left lung were firmly adherent as a result of an old pleural lesion. Both lungs were congested with blood and an excess of watery fluid could be squeezed out from both bases. There was no pus. There were numerous petechial and some ecchymotic haemorrhages of the pleural surfaces of both lungs.

#### *Alimentary system*

Tongue was thickly coated with a brownish fur. No abnormality could be found in the oesophagus or in the rest of the alimentary tract.

*Liver* This was normal in size but had the appearance of cloudy swelling on section.

*Spleen* This was enlarged about twice the normal size, was congested (and soft). No haemorrhages were seen.

*Kidneys* Both appeared normal.

*Brain* Appeared congested and oedematous. No petechial haemorrhages of brain or meninges were seen.

#### *Histology*

*Lungs* Haemorrhagic bronchopneumonia.

*Heart* Cloudy swelling of the myocardium and arteriolitis, with perivascular infiltration.

*Brain* Engorgement of small vessels. No perivascular or focal infiltration. No rickettsiae were found in Giemsa stained sections.

#### TREATMENT

Complete rest, maintenance of nutrition, symptomatic treatment and treatment of concurrent diseases was carried out as far as possible with the facilities available in an isolation centre.

Para-aminobenzoic acid was given to a series of six cases. All recovered, but the series was too small to be of significance. An initial dose of 6 grammes by mouth was followed by 2 grammes 2-hourly till the temperature had fallen to normal. The powder was kindly supplied by Brigadier FINDLAY.

#### MORTALITY

Of the total clinical cases discovered, thirty-two (25 per cent) died. Death occurred at any stage of the illness from the 5th to 27th day. The temperature always became subnormal before death.

#### PROGNOSIS

Patients who failed to rally after the temperature reached normal had a very bad prognosis. All patients developing coma eventually died. In those

was 103° F and remained steady throughout the illness. On 7th September, in the evening, finding no amelioration, the patient decided to come down to Nicosia. When the writer saw him temperature was 103° F and the pulse-rate 120. Headache by this time was very severe and intractable with congestion of conjunctivae. There was pronounced depression, insomnia, epistaxis and dry cough. The patient presented a well marked degree of toxæmia but his mental condition remained fairly normal throughout the illness without any delirium. He answered questions with lucidity. In the chest there were signs of diffuse bronchitis. The heart sounds were feeble but no bruits were heard. The urine contained traces of albumen. No splenomegaly or hepatomegaly were found. On the lower lip there was a sloughing ulcer. A sparse macular rash, comprising four or five spots on the abdomen, was seen.

During the next 3 days the rash, mainly present on the abdomen, also involved the whole trunk and limbs, including the soles, palms and the face. The rash was now maculo-papular originally of red colour changed to dark bluish-red, not disappearing on pressure. Some spots were very raised, notably on the wrist, fingers and face, while others on the soles, palms and limbs consisted rather of purpuric patches. On 10th September the rash began to fade and on 11th September there was only apparent faint subcuticular mottling which lasted for about 12 days. During the following days the general condition remained unchanged. The temperature was still 103° F the pulse-rate 120 the headache still severe and intractable insomnia and epistaxis continued. On 15th September there was slight improvement, and next day the temperature became normal. The patient had a rapid convalescence.

#### LABORATORY INVESTIGATIONS.

Blood culture on 10th September remained sterile.

No malaria parasites were found in blood-slides.

Widal reaction negative on two occasions.

Weil-Felix reaction Proteus X19

On 10th September 1/120

On 12th September 1/500.

On 17th September 1/1,250

The X-ray of the chest showed a completely normal chest film.

#### COMMENTARY

The clinical picture and the rising titre of the Weil-Felix test had left no doubt that the case was one of the typhus group. The diagnosis of this case as one of the typhus group appears to be obvious in retrospect but it was difficult at the time as no other cases of typhus were present in the Colony. The continued pyrexia, with epistaxis and the sparse rash, although not typical at the beginning led one to suspect typhoid fever.

The tick-exsthal agglutination and complement fixation test the most reliable method for differential diagnosis of the typhus group of fevers were not available. But the fact that the rash involved the soles, palms and face and was distinctly raised on certain parts of the body together with the absence of mental disturbances, and especially the presence of a primary ulcer led to the diagnosis of *fièvre boutonneuse Rhéopcephal sanguineus* the common vector in this type of typhus is found on practically every dog in Cyprus.

## OBSERVATIONS ON THE ACTION OF PALUDRINE ON MALARIAL PARASITES \*

BY

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AND

Q N ERCOLE, LIEUT., A.A.M.C

(From the L.H.Q. Medical Research Unit, Cairns, Australia)

### I—THE ACTION OF PALUDRINE ON *PLASMODIUM VIVAX*

#### 1 EFFECTS OBSERVED ON TROPHOZOITES

In blood films taken from patients before they received any therapy, it was observed that the nucleus of the full grown trophozoite divided first into two sharply defined masses, which usually moved well apart, before dividing repeatedly until a total of from eight to twenty-six discrete daughter nuclei were formed. It was not until nuclear divisions had proceeded considerably that the small particles of pigment began to coalesce, and it was only when actual merozoite formation was occurring that they became aggregated into the large, rounded central mass so characteristic of the mature schizont or rosette.

In contrast to the above series of changes, various abnormalities were noted when paludrine was given. These were observed in blood films taken at 2 or 4-hourly intervals from two patients, and in films taken at longer intervals from several other patients.

The results of the observations on one patient are given in some detail, as they are typical of what was observed in all. In this patient, Bas, two

\* The observations recorded here were made while the authors were members of the L.H.Q. Medical Research Unit, Cairns, Queensland, and have been briefly reported by FAIRLEY *et al* (1946a, 1946b). The work was carried out in 1945-46 at the same time as Capt R. H. BLACK'S *in vitro* experiments on the effect of paludrine on *Plasmodium falciparum* (BLACK, 1946). Our thanks are due to Brig N. HAMILTON FAIRLEY, Director of Medicine, A.M.F., for permission to publish, in its present form, and to the C.O. of the Unit, Lt-Col C. R. BICKERTON BLACKBURN, for his stimulating criticism.

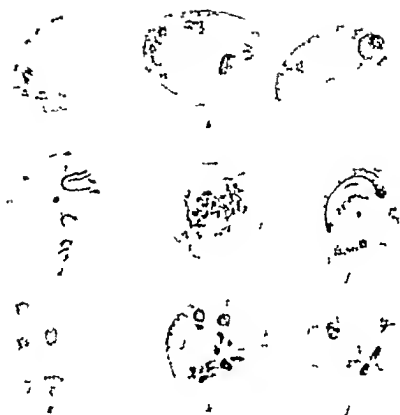
groups of parasites were present before treatment began, one group consisting mainly of young ameboids, and the other of large trophozoites and schizonts. The fate of each group was followed through until they disappeared. Parasite counts were made at 2-hourly intervals for the first 2 days, and subsequently at longer intervals. They showed a steady but slow decline. For the sake of brevity only the 8-hourly counts for the first 3 days and 12-hourly counts for the next 4 days are shown in Table I. Normal gametocytes were recognizable on the 6th day of therapy. On the 7th and 8th days only abnormal forms which were probably disintegrating gametocytes were seen. As it was impossible to identify with certainty all the forms seen, they are included under total parasites in Table I. No changes were seen which could be

TABLE I.

PARASITE COUNTS AT INTERVALS DURING THE ADMINISTRATION OF PALUDINE.  
PATIENT—B.B.

Day of therapy.	Hours after first paludine.	Total parasites per smear.	Male gametocytes per cent.	Forms recognized.				
				Ameboids.	Large trophozoites.	Schizonts.	Gametocytes.	Degrading parasites.
1	0	8,400	144	+	+	+	+	
	8	6,600	40	+	+	+	+	+
2	16	6,100	248	+	+	+	+	+
	24	4,000	120	+	+	+	+	+
	22	2,620	170	+	+	+	+	+
3	40	1,390	160	—	—	—	+	+
	48	900	80	—	—	—	+	+
	56	620	160	—	—	—	+	+
4	64	800	170	—	—	—	+	+
	72	840	180	—	—	—	+	+
5	84	440	160	—	—	—	+	+
	96	300	80	—	—	—	+	+
6	104	110	40	—	—	—	+	+
	120	120	10	—	—	—	+	+
7	122	40		—	—	—	—	+
	148	40		—	—	—	—	+
8	168	4		—	—	—	—	+
	172	—		—	—	—	—	—





*P. vivax* TROPHOZOITES AND SCHIZONTS FROM PATIENT BAE DE RU ALLDRIE K. THOR, PA.

- (a) Large trophozoite 4 hours after beginning therapy
- (b) Early schizont 4 hours after beginning therapy
- (c) Large trophozoite 30 hours after beginning therapy
- (d) Schizont 10 hours after beginning therapy
- (e) Schizont 8 hours after beginning therapy
- (f) Schizont 10 hours after beginning therapy
- (g) Schizont 12 hours after beginning therapy
- (h) Schizont 12 hours after beginning therapy
- (i) Schizont 10 hours after beginning therapy

(a), (b) and (c) represent the maximum development seen in those parasites which were young amoeboids when therapy began.

(d) — (i) represent the changes undergone by those parasites which were pre-schizonts and schizonts when therapy began.

attributed to the direct action of the drug on gametocytes their disappearance seemed more probably due to a natural process of death and disintegration. In films made soon after beginning treatment occasional merozooids were seen which showed abnormalities such as fragmentation of the cytoplasm or premature clumping of the pigment. However only a small minority of the merozooids showed these changes and it is doubtful if they could be ascribed to the action of the drug. The majority of the merozooids grew at a normal rate and reached full size at the expected time differing from normal parasites of like age only in a tendency for the pigment to be in larger particles and for it to be arranged peripherally (Plate Figs *a* and *c*). At this point however normal development ceased and although the nucleus might divide once (Plate Fig *b*) this was never followed by an orderly series of further divisions. On the contrary the chromatin seemed to break up and fall into irregular diffuse masses.

The second group of parasites—the large trophozoites and schizonts which were present at the beginning of therapy—showed these changes in 4 to 6 hours (Plate, Fig *e*). After 10 hours the chromatin appeared in peculiar twisted coils and irregular lumps (Plate Figs *d* and *f*). After 12 hours many parasites were seen with chromatin and pigment extruded from the cytoplasm (Plate Fig *g*). Some schizonts were represented by a collection of fragments of cytoplasm and chromatin while lumps of pigment might be attached to either, or free in the red cell (Plate Figs *h* and *i*). Occasionally all cytoplasm disappeared leaving only coils of swollen chromatin. Normal merozoites were not seen and new rings did not appear in the circulation.

## 2. EFFECTS OBSERVED ON GAMETOCTES

### (a) MORPHOLOGY AND NUMBERS

Paludrine had no recognizable effect on the morphology of *P. vivax* gametocytes in blood films stained with Leishman or Giemsa. In wet preparations made from 1 to 5 days after the beginning of therapy many gametocytes appeared quite normal with pigment in characteristically rapid motion. Exflagellation, with the formation of vigorous microgametes, was observed in one case on the 5th day of therapy (0.3 gramine daily) and in another on the 4th day of a higher dosage (1.0 gramine daily).

Paludrine certainly did not prevent newly formed gametocytes from entering the circulation as it was not unusual to observe an increase in numbers during the 2nd and 3rd day of therapy. While this phenomenon is commonly observed in patients on standard courses of quinine, atabrin and plasmoquine or intensive debtrin therapy it has been more frequently recorded and more marked in degree in patients on paludrine than in any other form of therapy including partial treatment with quinine or atabrin. (These results will be reported in a later paper.)



Counts of mature male gametocytes, which can be recognized with considerable certainty in thick films are more reliable than total counts, since the resemblance of large trophozoites to female gametocytes is confusing. In Table II the male gametocyte counts from three patients on paludrine therapy are set out.

TABLE II.  
COUNTS OF MALE GAMETOCYTES IN THICKS THE 1ST  
WITH ALUDRINE.

Day of therapy	Male gametocytes per c.mm.		
	STEV	CLA	STL
1	240	550	140
2	940	400	300
3	1 100	1,500	1,240
4	1 320	1 100	90
5	510	240	120
6	200	—	15
7	—	—	—

#### (b) DEFECTIVITY

In mosquitoes fed on gametocyte carriers after a single dose of 0.15 gramme or 0.2 gramme exflagellation, fertilization and vermicle formation occurred normally. The vermicules penetrated the gut wall and encysted. With one exception development in every batch of mosquitoes was arrested at this point, and 7 weeks after feeding, when the cysts should have been mature, all that could be found in the gut wall were small shrunken cysts containing a few clumps of pigment. In the exception referred to, mosquitoes were fed on Stev 4½ hours after his first dose. In about 50 per cent. of those dissected some cysts had grown a little, the two largest were measuring 35 25 and 22 20 (*i.e.* approximately half-grown). No further growth took place, the cysts ultimately became shrunken, and some became chitimized. No sporozoites were formed.

In mosquitoes fed on the 2nd day of therapy development occurred normally up to the vermicle stage, but penetration of the gut wall and formation of oöcyts was observed only once. Here again no sporozoites were formed.

Exflagellation and fertilization were observed in gut smears from mosquitoes fed on three patients on the 3rd day and in one patient on the 4th day of therapy but vermicules were not detected and no cysts appeared in the gut wall.

The results of feeding mosquitoes on patients with paludrine in the blood stream are set out in Table III.

TABLE III  
INFECTIVITY TO MOSQUITOES OF *P. vivax* GAMETOCYTES IN PATIENTS BEFORE AND AFTER  
THE COMMENCEMENT OF PALUDRINE THERAPY

Name	Time in relation to first paludrine	Amount of paludrine taken Progressive total (gramme)	Gametocytes per c.mm	Ex-flagellation	Vermiculation	Gut rate (per cent)	Average number of cysts per gut	Sporozoite rate (per cent)
Man	D—2 days	—	190	+	—	—	—	—
	D+4 hrs	0.15	700	++	+	100	50	90
	D+1 day	0.45	1,470	++	++	100	60	—
	D+2 days	0.75	820	++	—	—	—	—
	D+3 days	1.05	800	—	—	—	—	—
Cla	D+4 hrs	0.15	1,140	+++	+++	100	35	—
	D+9 hrs	0.3	—	++	++	100	20	—
	D+25 hrs	0.45	1,120	++	++	—	—	—
	D+34 hrs	0.6	—	++	—	4	—	—
	D+2 days	0.75	3,400	++	—	—	—	—
	D+3 days	1.05	2,700	++	—	—	—	—
	D+4 days	1.2	050	+	—	—	—	—
Harn	D—2 days	—	310	+	+	50	5	80
	D—1 day	—	300	+	+	100	10	100
	D—1 hr	—	370	++	++	93	4	93
	D+4 hrs	0.15*	330	++	++	70	3	—
	D+10 hrs	0.3	?	++	++	37	—	—
	D+2 days	0.9	?	+	—	—	—	—
Stev	D+4½ hrs	0.5	1,040	++	++	95	30	—

\* Some of dose vomited D = Day of infection

### 3 CONCLUSIONS

- 1 *P. vivax* trophozoites may grow to full size in the presence of paludrine. Its lethal action is exerted only upon the dividing nucleus, which is rapidly destroyed.
- 2 Paludrine does not directly inhibit the formation of gametocytes, nor influence the morphology of those already formed, nor does it prevent fertilization in the gut of the mosquito.
- 3 Gametocytes taken up by mosquitoes may reach the stage of encystment, but, owing to the persistent effect of the drug, all cysts eventually die. Complete sterilization of the gut infection in mosquitoes occurs after 0.15 gramme paludrine has been administered to the carrier.

## II THE ACTION OF PALUDRINE ON *PLASMODIUM FALCIPARUM* GAMETOCYTES.

### I EFFECTS ON GAMETOCYTES ALREADY PRESENT IN THE BLOOD.

Paludrine (0.1 or 0.3 grammes daily), given when gametocytes were already present in the blood had no effect upon their numbers or morphology. On the 1st day of therapy they behaved normally. In the mosquito, exflagellation and fertilization occurred. Vermicules were formed which were able to penetrate the gut wall and form cysts. These cysts however never grew and gradually shrivelled and disappeared or were represented by small chitimized spots. Complete sterilization of the infection occurred in mosquitoes fed 1 hour after taking the first dose of 0.1 grammes paludrine (Table IV).

On the 2nd day of therapy the gametocytes lost their power of development to the cyst stage. This loss was evidently not due to any irreversible

TABLE IV

EFFECT OF PALUDRINE ON NUMBERS AND EFFECTIVITY OF *P. falciparum* GAMETOCYTES.  
TIENTSIN—SEA.

Time in relation to drug.	Dose (grammes)	Progressive total (grammes).	Gametocytes per mm.	Gut infection (per cent).	Average number cysts (per gut).	Sporozoite rate (per cent).	Gland infection
D - 1 hour	—	—	1100	91	24	83	Heavy
D	0.1	—	—	—	—	—	—
D - 1 hour	—	0.1	1160	79	7	nil	—
D + 3 hours	—	0.1	1180	89	8	—	—
D - 5	—	0.1	1100	43	4	—	—
D - 7	—	0.1	1100	70	3	—	—
D + 25	0.1	0	790	30	—	—	—
D - 3 days	0.1	0.3	430	nil	nil	—	—
D + 3	0.1	0.4	48	—	—	—	—
D + 4	0.1	0.3	320	—	—	—	—
D + 5	0.1	0.0	320	—	—	—	—
D + 6	0.1	0.7	340	—	—	—	—
D + 7	0.1	0	700	—	—	—	—
D + 8	0.1	0.9	220	—	—	—	—
D - 9	0.1	1.0	200	—	—	—	—
D - 10	0.1	1.1	190	—	—	—	—
D - 11	0.1	1.2	—	—	—	—	—
D - 12	0.1	1.3	10	—	—	—	—
D + 13	0.1	1.4	90	—	—	—	—

change in the gametocytes, since, in some patients, they persisted in considerable numbers until the paludrine had been eliminated, and it was found they could infect mosquitoes again.

The sequence of events was as follows when mosquitoes were fed on gametocyte carriers 2, 4, 5 and 6 days after ceasing therapy (0.3 gramme daily for 10 days) no infection was recorded. On the 7th, 8th and 10th days cysts formed, but failed to grow. The final stage of the infection in mosquitoes fed on the 10th day resembled that seen on the 1st day of therapy, i.e., death and shrivelling of the small cysts. On the 12th day and thereafter cysts developed normally and sporozoites reached the salivary glands. Apparently traces of paludrine sufficient to kill the parasite were still present on the 10th day, but had disappeared on the 12th day after therapy.

Details of the sequence of events in three patients are given in Table V

TABLE V  
INFECTIVITY OF *P. falciparum* GAMETOCYTES AFTER CEASING PALUDRINE THERAPY

Patient and dosage	Days from ceasing drug	Gametocytes per c mm	Gut infection (per cent)	Average number cysts per gut	Sporozoite rate (per cent)	Gland infection
Sha (0.1 gramme daily for 10 days)	2	2 000	Nil	—	Nil	Heavy
	3	4,500				
	8	3 000				
	9	2 400				
	15	1,000				
Hoe (0.3 gramme daily for 10 days)	8	320	50	2	No record*	Heavy
	10	200				
	12	220				
	13	200				
	14	200	68	2	Nil	—
	16	200	85	6		
	20	240	69	7		
	21	150	57	7		
Wil (0.3 gramme daily for 10 days)	12	120	50	4	No record*	Medium
			30	2		
				2		
				2		
		640	100	26	87	Light
	13	700				
	15	530				
	16	240				
	17	210	94	20	No record*	Medium-heavy
			20	25	64	Light
			20	20	57	Medium-heavy

\* No mosquitoes survived for gland dissections, but cysts grew normally

The rate of recovery of infectivity varied with the amount of paludrine given the smaller the dosage the more rapid the return to full infectivity. There was some biological evidence that a single dose of 0.1 gramme was completely eliminated in 4 days (possibly earlier), and it was certainly eliminated in 6 days. When however the dose of 0.1 gramme was repeated three times in 1 day there was evidence that sufficient persisted to prevent complete development on the 7th day and possibly also on the 8th and 9th days, but had disappeared by the 10th day (See Table VI.)

TABLE VI.

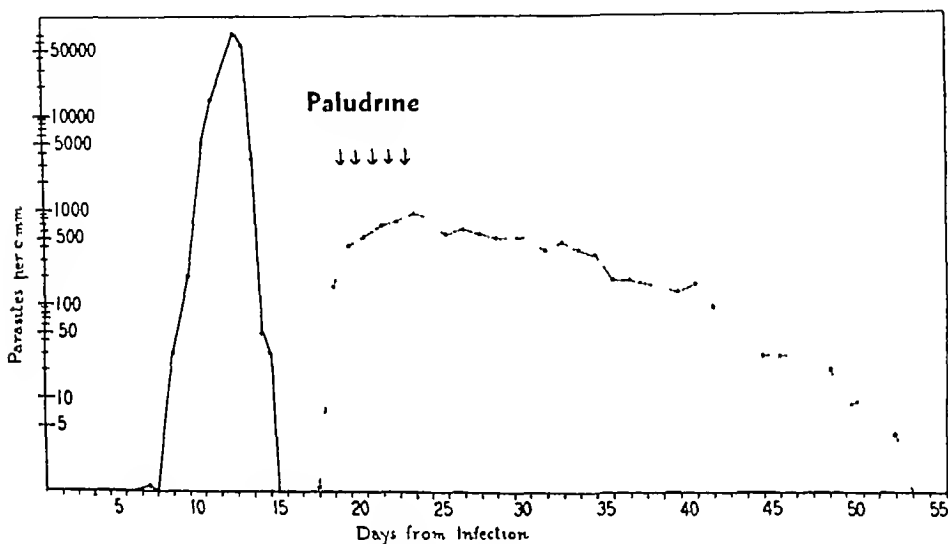
INFECTIVITY OF *P. falciparum* GAMETOCYTES APPEARING IN THE CIRCULATION AFTER THE ADMINISTRATION OF 0.1 OR 0.3 GRAMME PALUDRINE.

Patient and total dosage.	Days after drug	Gametocytes per cent.	Gut infection (per cent.)	Average number cysts per gut.	Sporozoite rate (per cent.)	Gland infection.	Remarks
Man. (0.1 gramme)	4	1,100	34	7	8	Medium Heavy	
	6	4,700	100	30	100		
	8	2,800	100	80	100		
Boy (0.1 gramme)	6	180	100	12	100	Medium Heavy	
	7	880	90	50	80		
	8	1,000	100	84	95		
	11	1,280	75	22	71		
Boy (0.3 gramme)	3	1,800	88	23	Nd	—	No growth of cysts.
	4	1,750	84	20	—	—	Slight growth of cysts
	5	1,780	85	88	—	—	A few cysts grown to full size
	6	1,370	90	100	No record	—	Slight growth of cysts
	7	1,400	100	80	Nd	—	Slight growth of cysts
	8	600	83	44	No record*	—	Slight growth of cysts
	9	870	100	42	No record	—	A few cysts grown to full size
	10	1,800	108	67	100	Light Medium-heavy	
	11	940	100	110	100		

N mosquitoes survived for gland dissections.

## 2 EFFECT ON GAMETOCYTE PRODUCTION

Gametocyte production was apparently not checked by paludrine, unless it was given very early in the attack, presumably before gametogeny had begun. The duration and height of the gametocyte wave could be correlated with the duration and height of the preceding trophozoite wave, in the same way that these waves can be correlated when atebirin is used to control the clinical attack. The gametocyte wave conformed to the normal type in all cases studied (see Graph).



GRAPH Trophozoite and gametocyte waves in patient Ken with sporozoite-induced falciparum malaria, treated with paludrine 0.3 gramme daily from the 13th to the 26th day. Trophozoites, continuous line, gametocytes, broken line, arrows indicate times when mosquitoes were applied. No infection was obtained in any of them.

## 3 THE EFFECT ON THE PARASITES OF PALUDRINE INGESTED BY INFECTED MOSQUITOES

An experiment was carried out to test the effect on subsequent infection of introducing paludrine along with infective gametocytes into the mosquitoes' stomachs. Mosquitoes were allowed to engorge partially on a patient who had no parasites in the blood and who was taking 1.0 gramme paludrine daily. Each mosquito was transferred to, and allowed to complete its feeding on, a patient with gametocytes in the blood. A control batch of mosquitoes was fed only on the second patient. Cysts in the control batch of mosquitoes grew normally and a heavy gland infection occurred. In the test series, cysts formed, but died without growing and no sporozoites were ever detected. The average

The rate of recovery of infectivity varied with the amount of paludrine given, the smaller the dosage the more rapid the return to full infectivity. There was some biological evidence that a single dose of 0.1 gramme was completely eliminated in 4 days (possibly earlier), and it was certainly eliminated in 6 days. When, however the dose of 0.1 gramme was repeated three times in 1 day there was evidence that sufficient persisted to prevent complete development on the 7th day and possibly also on the 8th and 9th days, but had disappeared by the 10th day (See Table VI.)

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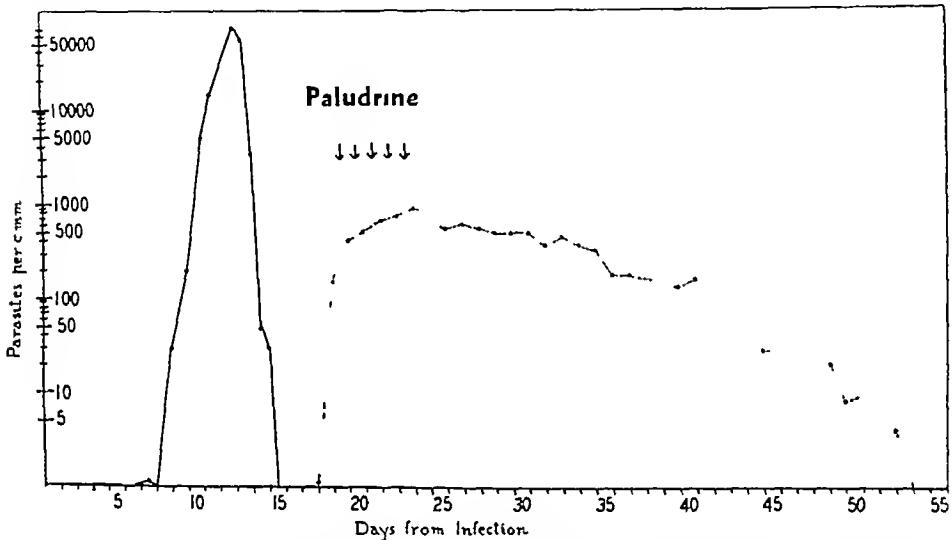
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Patient and total dosage.	Days after drug.	Gametocytes per c.mm.	Gut infection (per cent.).	Average number cysts per gut.	Sporozoite rate (per cent.).	Gland infection.	Remarks
Man. (0.1 gramme)	4	1,100	34	7	8	Medium Heavy	
	6	4,760	100	34	100		
	6	3,400	100	40	100		
Boy (0.1 gramme)	4	140	100	12	100	Medium Heavy	
	7	440	94	20	20		
	8	1,900	100	24	24		
	11	1,280	23	22	71		
Sib. (0.3 gramme)	3	1,800	80	22	Nd	—	No growth of cysts.
	4	1,780	24	20		—	
	5	1,700	66	60		—	Slight growth of cysts
	6	1,375	90	190	No record	—	A few cysts grew to full size
	7	1,400	100	20	Nd	—	Slight growth of cysts
	8	800	23	44	No record	—	Slight growth of cysts
	9	875	100	42	No record	—	A few cysts grew to full size
	10	1,000	100	67	100	Light Medium-heavy	
	11	940	100	110	100		

No mosquitoes survived for gland dissections.

## 2 EFFECT ON GAMETOCYTE PRODUCTION

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number of cysts formed in the controls was fourteen, and in the tests six and the corresponding sporozoite rates were 95 per cent. and nil.

Two experiments were carried out to test the effect of paludrine on cysts which were already growing normally. In these experiments the mosquitoes, already heavily infected, took a full feed from a patient on 1.0 gramme paludrine daily. In one experiment the cysts were 5 days old and in the other they were nearly mature. The sporozoite rate however was not influenced by the paludrine taken up by the mosquitoes. Apparently the drug did not penetrate the cyst, or if it did, it was not in sufficient concentration to prevent development, although innumerable nuclear divisions must have been occurring in the formation of sporozoites.

#### 4. CONCLUSIONS.

1. Paludrine in therapeutic doses does not inhibit the production of *P. falciparum* gametocytes and does not influence the numbers or morphology of those already formed.

2. When taken up by the mosquito, these gametocytes may reach the stage of encystation, but in the presence of paludrine development ceases at this point. Complete sterilization of the infection occurs in mosquitoes fed 1 hour after a dose of 0.1 gramme.

3. No irreversible change is produced in the gametocytes, since if they persist in the blood for a long enough period, they may regain their infectivity when the paludrine has been excreted.

4. Full infectivity had returned on the 12th but not on the 10th day after completing a course of 0.3 gramme daily for 10 days.

### III. THE ACTION OF PALUDRINE ON *PLASMODIUM MALARIAE*.

Observations were made on three patients treated with paludrine—one of them—St—had sporozoite-induced quartan malaria and the other two had been infected by blood inoculation. None of the patients had sufficient gametocytes to allow observations to be made on their infectivity to mosquitoes. Gametocytes occurred in small numbers during treatment and as they were only recognizable with certainty in thin films they have been included in the total parasite counts. Thick and thin films were made at 4-hourly intervals from two of the patients, and at daily intervals from the third.

#### St. (1.0 gramme paludrine daily for 14 days)

Before treatment began, the parasites present were mainly amoeboids and rings, with some early schizonts and some mature schizonts. Eight hours later mature schizonts were present, and one was seen with seven apparently normal merozoites. The rings and amoeboids were growing normally. At the 12th hour rings, amoeboids and pre-schizonts were present; at the 16th hour young rings, amoeboids and pre-schizonts and some mature schizonts were seen. The mature schizonts, however, were not entirely normal. The merozoites stained faintly and the cytoplasm was irregular in outline.

At the 20th hour amoeboids and pre-schizonts were present, and two abnormal mature schizonts were seen. One of these had three merozoites and the other only one. Some small rings were present. However, judging by later films none of the merozoites formed from the 16th to 20th hours continued its development. From the 28th hour onward, only large parasites were seen, among which the amoeboids, pre-schizonts and gametocytes appeared normal, while the schizonts showed nuclear changes similar to those described for *via* schizonts, i.e., swelling and distortion of the chromatin masses, with subsequent dissolution or extrusion of them from the cytoplasm. At the 72nd hour, the count dropped suddenly and thereafter only scanty parasites, which were probably degenerating gametocytes, were found. The blood was free from parasites on the 7th day of therapy.

TABLE VII  
PARASITE COUNTS ON PALUDRINE THERAPY

Days of therapy	Hours after first dose (approx)	Total parasites per c mm		
		HAR	STI	SCH
1	0	2 140	630	180
	8	2,300	500	
2	16	1 460	620	110
	24	1,500	530	
	32	1,020	480	
3	40	1 270	390	20
	48	1,100	480	
	56	800	400	
4	64	700	200	10
	72	710	80	
	80	450		
5	88	530	100	—
	96	300	30	
	104	190		
6	120	130	30	—
7	144	64	—	—
8	168	70	—	—
9	192	30	—	—
10	216	20		
	228	—		

Therapy STI 10 gramme paludrine daily for 14 days, HAR 10 gramme daily for 10 days

*Har* (0.3 gramme paludrine daily for 10 days).

The parasites in this patient had never been in phase, and some schizogony had occurred practically on every day of his infection. Before treatment began, the parasites consisted of well-grown rings, amoeboids, pre-schizonts and schizonts. After 4 hours, there was very little evidence of drug action, only one abnormal schizont was seen, in which the chromatin masses were vague in outline and stained palely. A few damaged schizonts were seen at 7 hours, but new rings had appeared in the circulation indicating that normal schizogony was occurring. They numbered about 90 per mm., and during the next 7 hours they increased to 390 per c.mm.

At 10 hours, early signs of nuclear destruction were apparent in many schizonts. At 14 hours, these changes were more marked, but one mature schizont with six normal merozoites was seen at this stage. The young rings, which were present in films taken from the 7th to the 20th hour could be followed through for 72 hours, when they were destroyed as schizonts.

There was gradual reduction in the total count. The blood picture, however, was almost unchanged up to the 60th hour: amoeboids, pre-schizonts, gametocytes and degenerating schizonts could be recognized in each film. From the 70th to 102nd hour only gametocytes and degenerating schizonts were recognizable and later than this all parasites were probably gametocytes, but, owing to their scarcity in the thin films, they were hard to study. The blood was not completely cleared of parasites until the evening of the 10th (and last) day of therapy.

In Table VII the counts from *Sci* and *Har* at approximately 8-hourly intervals are given. Daily counts from *Sch* whose parasites were too scanty for detailed study are given for comparison.

## CONCLUSIONS.

1. Paludrine does not prevent growth of *P. malariae* trophozoites. Its action is upon the early schizonts, which undergo the same type of degeneration as described for *P. vivax* schizonts.

2. *P. malariae* is less susceptible to paludrine than *P. vivax*: schizogony may occur up to the 20th hour after commencing therapy and on the lower dosage at least the resulting merozoites may grow to full size before being destroyed.

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# OUTBREAKS OF SPRUE DURING THE BURMA CAMPAIGN

BY

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(late Lieut.-Colonel, R.A.M.C.)

## INTRODUCTION

The object of this communication is to draw attention to outbreaks of sprue in British and Indian troops in the Burma campaign (1943-44) in which the association of this disease with dietary inadequacies is stressed

Sprue was first described by V. KETELAER in 1669, and again by HILLARY in 1766, who named the disease "diarrhoea alba." Its pathogeny has been elusive and numerous agencies have been put forward as causal and aetiological factors, infective, hormonal, inherent metabolic, etc. During recent years accumulating evidence has tended to indicate the malnutritional, or deficiency nature of the syndrome. ELDFERS (1919) and NICHOLLS (1918) first suggested that a vitamin deficiency was the cause of a sprue-like condition occurring in Indians. Following the discovery of liver-therapy for pernicious anaemia, it was found that its use in sprue was beneficial not only for the attendant improvement but for the other symptoms, also. CASTLE and RHOADS (1932) obtained improvement in sprue with liver extract and also with the yeast extract (marmite) which WILLS (1931) had shown to be curative in tropical macrocytic anaemia. RHOADS and MILLER (1934) demonstrated the value of parenterally administered whole liver extract (as opposed to refined concentrates) in cases refractory to previously beneficial sprue diets and coincidentally drew attention to the fact that whole liver and the recognized disease was thus strongly supported and parenteral crude liver coincidentally became established as the sheet-anchor in treatment. The factors implicated and present in crude liver were believed to be fractions of the vitamin B<sub>12</sub> complex. But the manner in which the deficiency arose was not clear, as parenterally administered liver could obviously remedy a dietary defect, gastric dysfunction or intestinal malabsorption, or relative degrees of the three in combination.

Other features of the sprue syndrome—diminished blood lipids, calcium, glucose, etc.—were also noted to disappear following liver therapy, a response shown by LEPORE (1941) to be associated with a return to normal intestinal absorption and function. The deficiency nature of sprue has been still further supported by radiological observations describing the so-called "deficiency pattern" of the mucosa, though this has been noted in other conditions in which there is interference with fat absorption. MACKIE, MILLER and RHOADS (1935), KANTOR (1939) and others have shown that the radiological or normal "herring-bone" appearance of the mucosa is restored following parenteral liver therapy. CRANDALL *et al* (1939) produced the deficiency pattern in dogs fed on a diet "deficient in vitamin B<sub>12</sub> complex."

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Many writers have given pre-eminence to antecedent intestinal disease as the dominant malabsorptive factor both in tropical and non-tropical varieties of sprue. BECKETT and HARDWICK (1940) ascribed the development of the sprue syndrome to "chronic jejuno-ileal insufficiency". LUSON BAKER (1941) wrote "that sprue is the expression of disease of the small intestine in man, and it now appears to be due to an avitaminosis through faulty absorption."

Hypothetically, if tropical sprue is a deficiency disease, the simplest way in which it could arise would be as the result of a dietary inadequacy of certain components of the vitamin B<sub>12</sub> complex. There is, however, little evidence in the literature of such an origin, and the evidence that the disease is of a deficiency nature lies mainly in therapeutic response to crude liver extract as observed clinically and radiologically. Perhaps the principal reason for this is that earlier reports, stating that no dietary deficiencies were discernible in the diets of those suffering from sprue, were written before the components of the vitamin B<sub>12</sub> complex had been identified and when little was known of their function in metabolism or even an approximate estimate of human requirement had been assessed.

A syndrome occurring in India with sprue-like features has long been known and has recently been re-described by NARITA (1943), CHANDRA and RAI CHAUDHURI (1944), and COOK (1944). It is characterized by glossitis, pale, watery diarrhoea, wasting, macrocytic anaemia, lowered blood calcium and blood lipids, and low or flat glucose tolerance curves. Malaria and dysentery are stressed as important contributory factors. These authors are agreed that the disease is of dietary origin and that vitamin B complex deficiency is the predominant factor both in aetiology and therapy. Though Cook believes that the syndrome is allied to sprue, NARITA names the disease "para-sprue" and considers that the two conditions not only have different aetiologies but distinguishing clinical features: the severity in degree of symptoms, the watery nature of the stools, the lower faecal fat levels, and the need for less restriction in diet during treatment justify the isolation. In NARITA's opinion, of para-sprue as an entity distinct from true sprue. Perusal of these reports admittedly reveals anomalies in certain features of the syndrome (para-sprue) seen typically in the Indian, and fully developed sprue as described in the European: but the reasons for this basic differentiation would be more convincing if new aetiological and pathogenic factors pertaining to either disease had been advanced to explain why two supposedly distinct syndromes should exhibit the same chain of clinical, haematological and biochemical events. Whatever differences of opinion exist these reports provide undeniable evidence of disease at least sprue-like in character associated with dietary deficiency of vitamin B<sub>12</sub> complex.

As a result of wider knowledge of fat and carbohydrate metabolism and of the metabolic functions of certain vitamin fractions various hypotheses have been advanced to explain the pathogeny of sprue, notably that of STANLEY (1947) to which reference will be made later. These hypotheses still await confirmation, and aetiological it is necessary to revert to an earlier assertion that our belief, that sprue is the expression of a deficiency of certain and as yet unspecified components of the vitamin B<sub>12</sub> complex is based principally on therapeutic response.

The following report of three outbreaks of the sprue syndrome in South-East Asia, in epidemic proportions, is submitted in the belief that it affords evidence of tropical sprue arising as the direct result of a dietary deficiency of the vitamin B<sub>12</sub> complex. The personnel affected were British and Indian troops engaged in the Burma campaign of 1943-44 in three widely separated areas. The report is a résumé of several reports submitted officially by the writer at the time of the outbreaks to appropriate headquarters. Of particular importance is the fact that in the second outbreak not only British but Indian troops in the same area were coincidentally affected: the majority of both nationalities suffered from the milder form of the disease which corresponds in description to the condition referred to above as para-sprue but as an

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appreciable number of cases developed symptoms of such severity as to be clinically indistinguishable from true sprue it is believed that the condition was fundamentally the same throughout, and the reasons for this conclusion will be discussed later. The observations are inevitably almost entirely clinical. Adequate and comprehensive investigation of these cases, historically unique in number and nature, was not possible under the conditions prevailing in forward areas.

It may be of historic interest that the report of the first outbreak furnished in September, 1943, is probably the first recorded account of an outbreak of sprue occurring in British personnel. The three outbreaks occurred in areas in which the writer served, but cases were by no means confined to these localities.

Such aetiological conclusions as have been reached pertain only to the condition which, for want of a better term, is traditionally named "tropical sprue."

### OUTBREAK 1

In August and early September, 1943, twenty-five cases (British troops) were admitted with gastro-intestinal symptoms to an Eastern Command (India) military hospital, of which the first nineteen were the subject of a special report. Though variously diagnosed before admission as clinical dysentery, enteritis, steatorrhoea, etc., they were found to have certain sprue-like features and were segregated in a separate ward. It was found that they belonged to the same group of units and had all contracted the disease in the same seaboard area on the Bengal coast, none had developed symptoms in a hill station. Length of service in India varied from 10 to 16 months with an average of slightly over 1 year. Previous illnesses included malaria, four, bacillary dysentery, one, enteritis, one, dengue, one, heat-exhaustion, one. In no case was there a previous history of fat intolerance. The first symptoms had arisen 3 to 4 months earlier (April and May) with the exception of two cases in June and one in July, the intervening period having been spent with their units and at intermediate hospitals.

### CLINICAL FEATURES AND LABORATORY FINDINGS

- (1) Prodromata mental and physical lethargy and progressive muscular weakness, loss or deterioration of appetite with particular distaste for fatty foods, flatulent dyspepsia after meals.
- (2) Diarrhoea slight and irregular at first, increasing in frequency, most stools being voided between midnight and 8 a.m. and accompanied by excess of flatus, noted to be worse after fatty meals. Stools were variously described as "watery," "pale," "greyish," "putty-coloured," "frothy," "greasy" and "foul-smelling."
- (3) Sore-tongue complained of in all cases but not an initial symptom, average time of appearance was 3 weeks after onset of diarrhoea, soreness

was increased by smoking and hot foods at first, later by all foods. Glossitis was found in all cases and varied from slight ulceration at the tip and along the margins of the tongue to completely red glazed tongue with epithelial atrophy. Aphthous ulcers were present in four cases.

(4) Loss of appetite pronounced in twelve cases.

(5) Abdominal distension after meals and especially during the night hours out of proportion to amount of food eaten.

(6) Loss of weight in all cases and in excess of that expected by severity of diarrhoea the heaviest loss recorded was 49 lb. between 15th July and 31st August. Four cases could be described as emaciated.

(7) Pronounced muscular weakness and mental depression.

(8) Pallor or unhealthy sallowness with dryness of skin and loss of elasticity no rashes were present but two cases exhibited a generalized pigmentation which, at first sight, was suggestive of Addison's disease.

(9) Sensations of numbness, tingling, and "pins and needles" in fingers and toes in six cases, of which two complained additionally of cramps in the calf muscles and feet, and two had oedema of the feet and legs. Of these six cases five had signs of polyneuritis with absent or sluggish knee and ankle jerks, tender calf muscles and peripheral sensory disturbances.

(10) Low irregular pyrexia in ten cases.

(11) Blood pressure systolic and diastolic pressures subnormal in all cases, and below 100 systolic in three cases.

(12) Blood anaemia present in all cases, macrocytic in seven. R.B.C. counts varied from 2,100,000 to 4,000,000. W.B.C. lowered in six cases. No case showed a leucocytosis and no malarial parasites were found in any films.

(13) Gastric analysis (using 100 c.c. of 7 per cent. alcohol) normal acidity six hyperchlorhydria, five hypochlorhydria, two achlorhydria, five. Excess of mucus in fifteen cases (one case had a total acidity of 164 and free hydrochloric acidity of 8, the odour denoting an excess of organic acids).

(14) Blood-sugar (following 100 grammes of glucose material available for estimation of four cases only) flat curve, two low curve, one normal curve, one.

(15) Stools.

(a) Naked-eye appearance pale, watery greasy foul smelling and containing undigested food particles in the majority a few stools were of a pale, pasty or porridgey consistence early morning stools were the most copious none was large as on admission up to twenty stools per day were being passed. (After initial milk diet and chalk powder had been taken for several days all the stools began to assume a pale, porridgey consistence and increased in bulk as frequency diminished.)

(b) Microscopic excess of fat globules and fatty acid crystals with undigested food particles. Ova of *Ascaris lumbricoides* found in three cases no *Entamoeba coli* *E. histolytica* cysts or *Giardia lamblia* in any case.

(c) Culture (all stools cultured) no dysenteric, salmonella, typhoid group or other pathogenic organisms grown

(d) Faecal fat analysis (by District Laboratory, Calcutta) Seven cases analysed following 60 grammes of fat daily for 4 days (Table I)

TABLE I

Case	Total fat per cent (dried faeces)	Split fat per cent	Unsplit fat per cent
1	10.9	11.2	8.7
2	22.6	21.4	1.2
3	25.3	11.3	14.0
4	28.0	25.7	2.3
5	33.6	31.4	2.2
6	33.8	17.6	16.2
7	40.0	38.0	2.0

(16) Urinary nicotinic acid excretion (Urine analysed at the All-India Institute of Hygiene and Public Health, Calcutta) The four cases tested had previously subsisted for 4 days on a diet of 3 pints of milk, one egg, 1 pint of liver marmite soup. Subnormal excretion levels were recorded in all four cases.

#### VISIT TO AFFECTED AREA

The hospital cases stated that a similar condition to their own with diarrhoea as the dominant complaint had been widespread in their units. A personal visit to the area was made and the following information obtained —

(1) All the units in the area appear to have been affected in some degree whether in proximity or miles apart. The prevailing symptom had been diarrhoea which had reached its height in May and June. No infective source had been found by the authorities, nor did stool cultures support an infective origin. Unit medical officers confirmed that the diarrhoea had been acute or sub-acute, mostly pale and watery, but a percentage pasty and frothy. Blood had not been passed with the stools. Lethargy, weakness, nausea, flatulent dyspepsia and anorexia had accompanied the diarrhoea. The majority had recovered with an initial purge, rest, milk diet and kaolin or chalk powder. Severely affected cases had been sent to hospital. The men had spontaneously voiced their distaste for fatty foods and these were either voluntarily refused or omitted by medical instruction. Glossitis had been present in the severe cases only.

(2) Accurate figures for the whole area could not be obtained — the disease had arisen 4 months before the visit, different diagnoses had been made in various units and many of those mildly affected had not reported sick. Medical officers of three units were able to furnish figures of "pale diarrhoea" showing that 14 per cent, 45 per cent, and 60 per cent had suffered in some degree in



these three units. At least 800 in the area were known to have been affected and the actual figure may well have been twice this number.

(3) The local military authorities had considered that the outbreak was in some way associated with an excess of fatty and fried foods and these were restricted in diet schedules. There was a general complaint from hospital patients and troops in the area that not only had there been an excess of fatty foods, but that the tinned fats, especially butter, margarine and cooking fat, had been "queer, unwholesome" or rancid—a complaint too widespread to be ignored. So distasteful had these articles been that many groups of men had privately bought tins of butter and margarine from the local town stores. Enquiry at the central food depot (from which all units in the area were supplied) elicited that for some months much of the tinned food arriving had been unsatisfactory—many tins had exceeded the consumption expiry dates stated on the labels. "Blown" tins with bulging lids were common but were of course, never issued to units, but necessity demanded that tins from the same consignment, if normal to outward appearance, were issued. The contents of a few such "normal" tins taken from cases in which one or more tins were "blown," were sampled and found to have a mildly rancid flavour. (Several such tins were put on one side by the writer and after 2 months one tin of milk and one of margarine had developed "blown" lids.) The officers in charge of the depot ascribed this deterioration to imperfect tropical storage conditions.

(4) Accurate details of the rations consumed daily by the troops 4 months previously could not be obtained. Authorized ration scales were satisfactory but it is to be noted that under military conditions unavailability of standard fresh foods such as eggs, milk, meat and vegetables necessitates issues of tinned substitutes and these may also vary week by week—the diets as prescribed authoritatively and as issued and consumed may therefore bear little relationship. Nor at this time, was an assessment of the vitamin values of the multiplicity of the tinned foods available. It was known that eggs, milk, fresh meat and vegetables had been in short supply for several months, but there was no proof that the tinned substitutes issued had been deficient in vitamin values, especially in terms of vitamin B<sub>6</sub> complex components.

(5) None of the unit cooks appeared to have suffered from the disease.

#### RESULTS OF TREATMENT

Response of the mild cases in the area to rest, an initial purge, milk and chalk powder and abstinence from fats has been referred to. The more severe cases in hospital were treated as for sprue with complete rest in bed and a high protein, low fat, low carbohydrate diet, consisting initially of 3 pints of milk, 1 pint of liver marmite soup, crisp toast and fresh fruit juice daily with gradual additions of an extra pint of milk, one egg, thin porridge, farax, bernaix, bananas, jellys, biscuits, marmite sandwiches, minced under-done beef and liver and an additional egg. Parenteral crude liver extract was given to the four

most severe cases. Drugs included kaolin and other chalk powders in large doses, dilute hydrochloric acid, ascorbic acid and nicotinic acid tablets, and full doses of iron and ammonium citrate in the 3rd week. Parenteral vitamin B<sub>1</sub> (benerva) produced immediately beneficial results in those with signs of polyneuritis. All the cases without exception responded to treatment and the following observations are emphasized —

(a) Four cases had received sulphapyridine or sulphaguanidine before admission and two cases were given the former drug after admission, amelioration of symptoms was not in advance of those to whom the drug was not administered.

(b) It was soon apparent that rigid dietetic restrictions were essential and attempts to commence with a diet of higher level had to be discontinued owing to persistence of diarrhoea, nausea, distension and other symptoms. (This should be compared with the observation of NAPIER and CHAUDHURI that in para-sprue there is no need for the rigid dietetic restrictions imposed in sprue.) Unfortunately it was not possible to note the effect of a more liberal diet given coincidentally with parenteral liver extract.

(c) The limited amount of parenteral crude liver extract available was given to four cases in doses of 8 c.c. daily for 4 days, followed by injections on alternate days. The gain in weight of the cases on diet and oral therapy alone was 1½ to 3½ lb., during the second week no case gaining more than the latter amount. The four cases who received parenteral liver in addition, gained 6 lb., 7 lb (two cases) and 8 lb respectively with earlier cessation of diarrhoea and more rapid improvement in all other respects than the remainder.

(d) Reiteration is necessary of the observation that the initial treatment of rest, milk, kaolin and liver soup resulted within a few days in a change in the stools, which lost their pale, watery appearance and became less frequent, larger, putty-coloured and of a pasty consistence, the massive, pultaceous, early morning stool of the established sprue patient was at no time observed.

(e) At the end of five weeks' treatment further observation was cut short owing to the writer being posted to another hospital coincidentally with the transfer of many of the cases to a Base hospital. General improvement had been maintained in all cases, symptoms of glossitis, dysphagia and distension had abated or ceased and blood counts and haemoglobin had shown a satisfactory increase, twelve cases were passing only one stool daily. In only two cases had the normal increases in diet to be withheld owing to persistence of looseness of stools and other symptoms. The four severe cases who had received parenteral liver extract for 2 weeks were passing well-formed stools, had maintained a greater increase of weight, more speedy abatement of other symptoms and a more rapid approach to normal blood counts than those treated by oral methods alone, it had also been found possible to increase their diets to a higher level more quickly without ill effects. In short, the response to sprue treatment by oral methods was precisely of the same nature and degree as one would expect

pronounced and a few bouts of vomiting ensued. Following return to the area symptoms persisted and were now accompanied by painful flatulence after all meals and loss of weight. For about 3 months the two main meals of the day had been *dishes* prepared from bully-beef or tinned fish with dehydrated potatoes cheese pickles and tinned beans. H stated that the margarine was repulsive and most of the unit gave up eating it. Either ghi or Indian cooking oil was said to be used for frying and "everything fried in it had an unpleasant, sickly taste. His stools were described as pale creamy or yellow almost white when very liquid.

#### CASES TRANSFERRED TO BASE HOSPITALS.

Cases of severity were constantly being transferred from this forward area to Base hospitals. In a personal communication from the medical specialist of a military hospital in Calcutta it was stated that they were being diagnosed as sprue and that in many cases total faecal fat readings of over 50 per cent. with excessive splitting had been found.

#### THE DIETARY FACTOR.

Weekly ration schedules for a preceding period of 3 months were studied for both British and Indian troops and showed only minor variations. These schedules were drawn up in advance in accordance with the official "Scales of Rations and Supplies" (S.R.S.), the scale providing for the issue of standard articles (the supply of which often depended on local purchase and availability) and a table of substitutes for those standard articles which could not be provided. This method was found to be unsatisfactory and the S.R.S. were drastically amended in 1944. It will be readily understood that in a forward area the requisite supplies of fresh foods were at times impossible to obtain and their replacement by substitutes equal in essentials difficult to ensure. These weekly schedules were, therefore, prepared in advance to ensure the issue of the rations stated if available. They do not, however, accurately represent the actual issues. Officers at the supply depots supplied the following information: fresh foods, especially meat, eggs, fruit and vegetables, had been in short supply for months and the official policy had been to send the bulk of these articles to advanced troops in the forward zones, adhering as closely as possible to the full scale of rations authorized in the "S.R.S." In the Base area itself it had not been possible to issue fresh meat more than once a week (occasionally twice) for several months. Eggs had been unavailable in the Base for weeks on end. The issue of fresh fish was dependent on a catch in the local river—never sufficient to supply more than one or two units at a time. Only occasional issues of fresh milk and fowl had been possible. Fresh vegetables had been extremely scarce for months and of poor quality. Brinjals and pumpkin were the common ones issued if available, but in the main they had had to depend on dehydrated substitutes, including potatoes. In the case of Indian rations, eggs were not an authorized issue and fresh meat (mutton or goat) had been issued once weekly on the average. The above observations were in keeping with statements made by troops themselves and unit cooks, many of whom

were able to produce menus of actual meals cooked and issued during the preceding 2 months

Details of two specimen ration schedules are appended, one British and one Indian, giving the daily amounts of each foodstuff per man. These articles and amounts *as prescribed* are given in the tables, and the necessary deductions for unavailable articles, and revised values of riboflavin and nicotinic acid follow. Values for iron, sodium chloride and vitamins A and D have been omitted. The amounts of pyridoxin, biotin, pantothenic acid and other vitamin B<sub>2</sub> complex components present in the foods mentioned have not yet been so accurately assessed as to render tabulation possible (Table II, p 388, Table III, p 390)

#### DEDUCTIONS AND REVISED VALUES

The omission of eggs, fresh meat, fish and fowl from the British diet (Table II) would reduce the riboflavin value by 0.6 mg to a total of 0.99, and the nicotinic acid value by 13.5 mg to a total of 11.0 mg. Proportionate issues of the commonly substituted articles—corned beef (4 oz), tinned meat and vegetables (2 6/7 oz) and tinned fish (2 oz) would restore the values of riboflavin by 0.23 mg to a total of 1.22 mg, and nicotinic acid by 3.0 mg to a total of 14.0 mg.

The loss in cooking of riboflavin has been estimated at 10 per cent, and of nicotinic acid at up to 20 per cent. There is little loss of riboflavin during six months' storage but up to 20 per cent. may be lost through storage at 115° F for 8 months. To these losses must be added what is known as "plate wastage". If these losses are conservatively and inclusively assessed at 15 per cent, the approximate values of the daily food consumed, in terms of these components, would have been: riboflavin (1.22 mg less 0.18 mg) = 1.04 mg, nicotinic acid (14.0 mg less 2.0 mg) = 12.0 mg. It has been found that certain Indian canning processes resulted in a loss of up to 67 per cent of riboflavin. No deduction has been made for issues of dehydrated potatoes, and as the amounts of thiamin, riboflavin and nicotinic acid present in corned beef are a matter of disagreement (some authorities even stating that corned beef contains none of these factors), the figures given above probably still represent too high an assessment.

The normal (optimum) requirements of riboflavin and nicotinic acid for a diet of the above value, *i.e.*, 3,571 calories, under ordinary conditions would be approximately 3 mg and 20 mg respectively (National Research Council, U.S.A., 1941). As regards these two components, therefore, the diet over a period of months may be said to have contained minimal or border-line levels at which impaired vitality and a falling level of fitness might be expected to appear, if not frank signs of specific deficiency symptoms.

It has been generally assumed hitherto that a diet known to contain adequate amounts of thiamin, riboflavin and nicotinic acid also contains adequate amounts of the other components of the vitamin B<sub>2</sub> complex—biotin, pantothenic acid,

TABLE II  
 BRITISH TROOPS DAILY TOTAL  
 9th-12th May 1943.

Commodity	Quantity in oz.	Protein Grammes.	Fat Grammes.	Carbo-hydrate Grammes.	Calor. kcal.	Calcium Mg.	Vit. B <sub>1</sub> IU	Riboflavin Mls.	Nicotinic acid Mg.	C ascorbic acid.
Bacon (tinned)	1 3/7	4.4	14.3	—	183	4	80	0.18	0.4	—
Cherries	5/7	8.0	7.0	—	53	184	3	0.10	—	—
Beans (tinned)	1 1/2	2.8	—	5.6	37	4	9	0.01	0.3	1
Bread (white)	14	22	2.8	216.4	1022	56	70	0.14	2.6	—
Curry powder	1/100	—	—	—	—	—	—	—	—	—
Fruit (dates)	4/7	0.3	—	7.0	30	10	—	—	—	—
(tinned)	—	0.4	—	0.2	34	6	4	0.02	0	2
Fish	7/8	1.5	1.1	—	17	11	—	0.01	0.4	—
Eggs	4/7 No — 0.6 E.P.	1.7	1.4	0.1	2	8	7	0.03	—	—
Sardines	1/8	0.7	0.0	—	16	14	—	0.41	0.1	—
Jam	3/7	—	—	6.1	40	—	—	—	—	—
Golden syrup	3/7	—	—	11.4	68	0	—	—	—	—
Margarine	2 2/7	—	53.3	—	484	2	—	—	—	—
Meat (fresh)	8 = 6 E.P.	28.8	27.0	—	260	19	42	0.42	7.6	—
Fish	2 2/7 = 1.0 E.P.	5.8	0.3	—	40	13	11	0.07	1.1	—
Fowl	4/7 = 1 E.P.	10.2	4.0	—	76	6	14	0.04	4.6	—
Meat and egg	3 0/7	6.7	4.0	1.1	102	11	28	0.03	0.8	—
Milk (fresh)	6	3.4	6.0	7.2	10	404	24	0.4	—	—
Mustard	1/100	—	—	—	—	—	—	—	—	—
Onion	2/7	1.0	0.7	8.3	31	4	1	0.01	—	—
Rice	1 2/7	2.6	0.7	27.0	1	3	38	0.02	2.2	—
Onions	2	0.6	—	2.0	12	14	0	—	—	0
Pepper	1/100	—	—	—	—	—	—	—	—	—
Potatoes	1 = 0 E.P.	8.4	—	57.0	140	15	80	0.18	2.7	43
Salt	1/2	—	—	—	—	—	—	—	—	—
Sugar	3 1/2	—	—	0.1	37	—	—	—	—	—
Tea	1/2	—	—	—	—	—	—	—	—	—
Veg	8	4	—	0.4	3	4	41	0.08	0.8	58
Flour (bar)	1	2.3	0.3	21.0	94	0	8	0.01	0.3	—
Total per day		121.6	120.8	499.8	2,871	694	494	1.59	1.4	113

pyridoxin, etc. It is not unreasonable to assume that the reverse also applies and that, therefore, in the above ration issue there was a continued inadequacy of vitamin B<sub>2</sub> complex components in general. In a paper on "Nutritional Diarrhoea," AYKROYD and GOPALAN (1945) showed that whilst this condition responded to nicotinic acid, the treatment of concomitantly occurring cases of sprue by the parenteral administration of nicotinic acid and riboflavin produced no amelioration, although the response to whole liver was immediate. Other components of this complex are implicated in sprue. It is felt, however, that the inadequacy of riboflavin and nicotinic acid affords presumptive evidence of inadequacy of other fractions, especially as it is known that the usual sources of vitamin B<sub>2</sub> complex are precisely those foods which were so scarce—fresh milk, eggs, fish, liver, kidney and other fresh meats, fresh vegetables, potatoes, etc. Further, although the extent to which a deficiency of one component affects the activity of the others is not fully known, that such an interference does occur is likely in view of the close functional relationship of thiamin, riboflavin and nicotinic acid which recent research has shown to exist, particularly in their catalytic function of energy-transformation by oxidation.

### INDIAN RATIONS

#### DEDUCTIONS AND REVISED VALUES

Assuming that the Indian diet (Table III) was issued as prescribed, the following deductions are necessary. It was stated that the rice available for several months had been mainly parboiled and polished. The loss of riboflavin and nicotinic acid during cooking (where the cooking water is not consumed) has been assessed at 30 per cent in parboiled rice and 70 per cent in other rices, while the loss in other cooked foods is approximately 10 per cent. Allowing for a 50 per cent mean loss from rice, the riboflavin total would be reduced by 0.29 mg to 1.69 mg, and the nicotinic acid total by 3.3 mg to 16.9 mg. Storage and plate wastage would account for further losses. When meat was not available, tinned milk, or ghee and atta (or rice) were issued as substitutes. The issues of rice and atta varied and on days when rice was issued in excess of atta there would be smaller amounts of the components named. Some dals contain only small amounts of riboflavin and pyridoxin whilst no deductions have been made for the frequent issues of dehydrated potatoes and vegetables. If these further losses are collectively assessed at 15 per cent, the Indian diet would approximately contain riboflavin (1.69 mg less 0.25 mg) = 1.44 mg, nicotinic acid (16.9 mg less 2.5 mg) = 14.4 mg. The normal (optimal) requirements of such a diet of 4,032 calories are riboflavin 3.0 to 3.3 mg, and nicotinic acid 20.0 to 23.0 mg. The values calculated indicate a minimal or border-line amount of riboflavin and nicotinic acid. As in the case of British troops subsistence on such a diet over a period of months would be expected to result in a general deterioration of health and vitality with the possibility of specific deficiency symptoms appearing. It cannot be too strongly emphasized that in both British and Indian

TABLE III.  
INDIAN TROOP'S DAILY RATIONS.  
9th-18th May 1943.

Commodity	Quantity in oz.	Protein. Grammes.	Fat. Grammes.	Carbo-hydrate. Grammes.	Calor. kcal.	Calcium. Mlg.	Vit. B <sub>1</sub> . I.U.	Riboflavin. Mlg.	Nicotinic acid. Mlg.	C ascorbic acid
Rice	11	19.8	2.3	244.2	1,039	11	83	1.32	3.3	—
Arise	11	27.4	8.6	213.5	1,160	121	261	0.98	8.8	—
Dal	2½	14.7	1.1	24.0	221	23	96	0.17	1.17	—
Glu	½	—	58.8	—	812	—	—	—	—	—
Milk (fresh)	8	8.4	0.0	7.1	107	204	24	0.24	—	2
Onions	2	0.6	—	2.8	1	18	6	0.02	—	8
Potatoes	4-3 E.P.	1.8	—	19.2	83	0	23	0.08	0.8	15
Vegetables..	4	1.2	—	3.1	16	40	26	0.04	0.4	12
Dal (whole)	2	12.6	1.0	22.6	190	80	64	0.04	2.8	—
Sugar	2½	—	—	83.0	321	—	—	—	—	—
Meat (fresh)	1 5/7-1 2/3 E.P.	8.1	8.7	—	77	4	9	0.08	1.6	—
Salt	½	—	—	—	—	—	—	—	—	—
Tea	½	—	—	—	—	—	—	—	—	—
Chilies	½	0.7	8	4.9	17	11	—	0.03	—	25
Garlic	1/8	—	—	—	—	—	—	—	—	—
Onion	1/8	—	—	—	—	—	—	—	—	—
Turmeric	1/8	—	—	—	—	—	—	—	—	—
Milk (skimmed)	3	7.2	7.6	9.9	139	249	18	30	3	—
Ground nuts	2-1 1/4 E.P.	11.2	18.8	3.1	72	24	117	0.11	0.8	—
Total per day		118.7	108.8	653.6	4,821	821	1,868	1.98	20.27	26

sufferers subsistence on the diets quoted was for a limited period only. Once symptoms had appeared appetite deteriorated—often rapidly—until the daily consumption was but a fraction of the values given in the appended scale of rations.

#### TREATMENT OF SEVERE CASES.

By arrangement, two severe cases, Q.M.S. M. and Gur B ( whose histories have been briefly recorded on p. 385) were transferred to the hospital where I was in charge of the Medical Division, and where sufficient parenteral liver extract for the thorough treatment of two cases was available. On first-stage sprue diet, large doses of kaolin and 10 c.c. parenteral crude liver daily diarrhoea ceased and both patients were feeding almost solid

stools at the end of the week, kaolin was discontinued on the 5th day. At the end of 2 months, both patients had fully regained their weight, had normal blood-counts and after subsisting on hospital convalescent diet without parenteral liver for 3 weeks had shown no tendency to relapse in spite of there being no restriction of fats. Both were now passing one normal solid stool daily. Q M S M proceeded to a convalescent depot. Gnr B, unfortunately, contracted severe Shiga dysentery and a recrudescence of his former symptoms was feared. On sulphaguanidine therapy he recovered rapidly from the dysentery without any complications and proceeded from convalescent diet to ordinary hospital diet without any signs of fat intolerance or other sprue symptoms. He was kept under observation for a further 2 months, during which time he subsisted on the R A M C unit rations (Field Service Scale) and remained perfectly well.

### APPARENT IMMUNITY OF FORWARD TROOPS

Freedom from the syndrome described by units in advanced positions has been referred to, in spite of their greater exposure and more arduous nature of their duties. The significance of this finding cannot be too strongly emphasized. These troops received the bulk of such supplies of fresh meat, eggs, fresh fruit and vegetables as were available, their ration issues approximating closely to the ration schedules, details of which have been given, and in which the vitamin B<sub>2</sub> complex values were appreciably higher than in the rations actually issued at the Base area. This observation is considered a strong indication that the additional fresh foods had given to the advanced troops a protective vitamin balance.

### OUTBREAK III

In May, 1944, an Indian Artillery Regiment was transferred to the northern area from the Arakan where the unit had been part of a division surrounded by the Japanese and for 6 months had been subjected to most arduous conditions, for the greater part of which period they had been dependent on air-borne supplies. After transfer to our northern area several men were admitted to hospital with sprue symptoms: glossitis, angular stomatitis, anaemia, pronounced muscular weakness, pale, watery stools, and severe loss of weight. I was allowed to make a personal inspection of the unit, the total strength of which was 985. The general air of apathy and listlessness was striking. Two hundred had mild symptoms, ninety, moderate, ninety-six were severely affected and were immediately admitted to hospital. (This number was later increased to 112.) Forty per cent of the unit were therefore obviously affected. The incidence was significant. The Bikaners numbered 235, of whom 141 (60 per cent) had the disease. The Ahirs comprised half the unit and of these 130 (26 per cent) were affected. The State of Bikaner was swept by famine and flood in 1940-41 and it was stated that the majority of the Bikaners were recruited shortly afterwards. Antecedent malarial incidence was also highest in the Bikaners and Ahirs in that order, and their general physique was not so good as in the Punjabi Musselmen who suffered least from sprue symptoms and in whom the malarial incidence was lowest.

*Duration of Symptoms*—The average duration of symptoms before the first admissions to hospital was only 3 to 4 weeks. Malnutrition must have been



present, however before the advent of glossitis diarrhoea, etc., as the weight loss in the severe cases was high, one man having lost 40 lb and the majority over 2 stones (28 lb).

### THE DIETETIC FACTOR.

Issues of food had been carried out under severe emergency conditions and no accurate records existed. The daily food issues appended below (Table IV) are based on the statement of four officers of the unit, and approximately represent the daily allowance over a period of at least three months. Atta and rice were issued in daily amounts of 10 oz. per man the assessment assumes an average of 5 oz. of each of these articles over the period. The amounts of

TABLE IV  
FOUR V TROOP'S DAILY RATIONS (MOUNTAIN ARTILLERY REGIMENT)

Commodity	Quantity in oz.	Protein. Grammes.	Fac. Grammes.	Carbo- hydrate. Grammes.	Calories.	Calcium. Mg.	Vitamin B <sub>1</sub> IU	Riboflavin. Mg	Nicotinic acid Mg
Biscuits (Bakaspa)	4	10.4	17.2	80.0	320	3.3	90	0.1	0.8
Atta	5	17.0	2.6	102.3	406	2.5	51	0.17	7.0
Rice	5	9.0	1.6	111.4	465	5	40	0.10	1.8
Dal	3	14.3	2.1	49.3	91	14.1	153	0.22	1.8
Milk (tinned)	3	7.2	7.4	9.9	124	24.9	18	0.20	9.2
Ghi	—	—	23.0	—	456	—	—	—	—
Tea	1	—	—	—	—	—	—	—	—
Sugar	2½	—	—	23.0	212	—	—	—	—
Total per day		81.0	23.1	415.9	2,634	472	218	0.81	11.1

sugar tinned milk and ghi are approximate. Salt, chillies, turmeric, etc., have not been included. The usual weekly extras had not been available. One orange or banana per man, and one ration of vegetable had been issued monthly. There was some doubt as to whether two, or three, issues of meat had been made in 6 months.

### REVISED VALUES THROUGH LOSSES.

It will be noted that the above rations contained no animal protein, fresh milk, eggs, fruit or vegetables. The small amounts of the two latter articles issued monthly and the two (or three) issues of meat over a period of 6 months are negligible. Seventy four per cent. of the diet was carbohydrate. The only source of vitamin C was dal and the daily intake would vary from 3 to 9 mg according to the type of dal. With regard to thiamin, riboflavin and nicotinic acid, making the same percentage deductions for losses in cooking as detailed

in Outbreak II, the above values would be reduced to thiamin, 1.3 mg, riboflavin, 0.78 mg, nicotinic acid, 9.3 mg

Plate wastage, issues of rice in excess of atta, or poorer quality dals would still further reduce these amounts. Further reduced intake through eventual loss of appetite was an additional factor and was described as pronounced. The optimum requirements for a diet of the above calorie value are thiamin, 1.5 mg, riboflavin, 2.2 mg, and nicotinic acid, 15 mg (National Research Council, U.S.A.)

#### URINARY EXCRETION OF NICOTINIC ACID

Unfortunately the unit was not seen and these tests not made until 10 days after its arrival, during which time the troops had been subsisting on the full and more generous scale of rations now available to them, including fresh meat, milk, fruit, vegetables and larger daily issues of atta, rice and dal. Allowance must be made for this in interpreting the readings obtained. Since the entire unit had previously received the same low diet, subnormal nicotinic acid excretion levels might reasonably have been expected even in the apparently fit members. It was of particular interest to note, however, that whilst the latter and those with only mild symptoms were eager and able to take the full diet now available, those with severe symptoms had lost their appetites to such a degree that they were unable to take advantage of the rations at their disposal.

Three groups of eight men each were selected for urinary analysis and the output of urine in 24 hours carefully measured. (The analyses were made by the All-India Institute of Hygiene and Public Health, Calcutta.)

- 1 Group A (eight cases) Apparently fit with no symptoms
- 2 Group B (eight cases) Mild symptoms, but without diarrhoea
- 3 Group C (eight cases) Severe symptoms, including diarrhoea

The results were as follows —

TABLE V

Group A		Group B		Group C	
Case	Nicotinic acid in 24 hours' urine Mg	Case	Nicotinic acid in 24 hours' urine Mg	Case	Nicotinic acid in 24 hours' urine Mg
1	4.1	1	5.5	1	1.4
2	5.1	2	3.1	2	1.9
3	2.3	3	2.1	3	1.7
4	7.5	4	3.1	4	2.7
5	3.7	5	4.5	5	2.2
6	5.0	6	1.2	6	0.9
7	5.6	7	4.3	7	2.0
8	8.7	8	3.4	8	0.7
Average	5.3	Average	3.4	Average	1.6

## STANDARDS FOR COMPARISON (INDIAN).

*Normal Subject*—Wheat enters, 6.77 mg. nicotinic acid in 24 hours. Rice enters, 3.15 mg. nicotinic acid in 24 hours.

The readings in Groups A and B are believed to be higher than would have been the case had the analyses been made 10 days earlier before the full ration scale had been issued. The lower readings in Group B, as compared with Group A, are significant considering that there was no loss due to diarrhoea, a factor which must have played some part in the low readings in Group C in addition to any dietetic deficiency. The striking feature of the results is that the nicotinic acid excretion levels were precisely parallel with the degree of clinical involvement in the three groups. Coincidental riboflavin analyses, unfortunately could not be made.

## RESPONSE TO TREATMENT

The unaffected and mild cases were retained in their unit lines where as much rest as possible was enforced. Fatty foods were eliminated or restricted and supplementary rations issued of fresh milk, marmite, eggs, meat and bananas and nicotinic acid tablets (three to six per man daily). Kaolin powder or prepared chalk and a fern-sulphate mixture were prescribed where needed by the unit medical officer. The retention of 112 cases in hospital reduced the strength of the regiment by one battery. The rest of the unit responded well to the above measures, proceeded 3 weeks later into action and acquitted themselves well.

The severely affected cases exhibited the sprue-like features described in Outbreaks I and II. Time and the urgency of the military situation precluded any delays through complex investigations and treatment was commenced immediately. Glossitis was present in every case. 5 per cent. had an angular stomatitis, and a macrocytic anaemia was present in 62 per cent. The stools were pale, watery and fermenting, occasionally of a pasty nature, in many frankly greasy and microscopically showed excess of fat-globules and fatty-acid crystals. Intestinal parasites were found in twelve cases and *E. histolytica* or cysts in five cases. The incidence of malaria was high—the entire unit had been taking suppressive doses of mepracrine for 6 months and these were continued for 3 weeks until general improvement was established. As twenty-two cases quickly developed malaria following stoppage of the drug all the cases were given a full malarial “blanket” treatment.

Treatment for the condition was commenced with 3 pints of milk, 1 pint of liver marmite soup and 10 c.c. of parenteral whole liver extract daily the latter being reduced to alternate days in the 2nd week. Milk puddings, minced meat, bananas, eggs, rice and chupatus were added later. Diarrhoea was controlled with large doses of kaolin or chalk powder. Compound vitamin tablets, now available, containing vitamin C and thiamin, riboflavin and nicotinic acid were given daily and mist. ferri et ammon. cit. in full doses during the 2nd week.

On this sprue regime response was satisfactory and immediate with amelioration of all symptoms. The highest gain in weight at the end of 8 weeks was 34 lb. and the majority at the end of this period were given special leave for

1 month to their homes before rejoining their unit. At the end of 3 months fifteen cases remained in hospital out of the original 112, these cases included pulmonary tuberculosis (one case), chronic bronchitis and emphysema, malarial relapses and ankylostomiasis. Eight cases responded very slowly with relapses of glossitis, and diarrhoea. They were the most debilitated, and continued anorexia and refusal to eat meat and other foods played a major part in their tardy progress.

*The Syndrome in Other Units*—It was found at a later date that at least three other units of the brigade to which the above mountain regiment belonged and which had been subjected to the same conditions in the Arakan suffered from the same syndrome.

## MISCELLANEOUS CASES

### 1 "K RATIONS"

During the severe jungle fighting which accompanied the Japanese retreat in 1944 a number of British troops were admitted to hospital suffering from bacillary dysentery, the majority giving a history of blood-stained stools, and positive cultures were obtained in 30 per cent of cases. Approximately 10 per cent suffered from sprue symptoms in addition, in these, prodromata, glossitis, pale stools, etc., had either been present before the dysentery, or the latter appeared to have precipitated these symptoms.

There was a tendency to ascribe the deficiency features to previous subsistence on "K rations". Although the latter were intended for emergency use for brief periods only, many of the troops had subsisted on them for up to 10 weeks. I do not believe that deficiency symptoms were due to vitamin inadequacies in "K rations". They were simply not eaten. After 1 or more weeks the monotony of eating the same articles, day after day, for each breakfast, dinner and supper, caused rapid deterioration of appetite until the rations were either mainly discarded or given to others. Previously the affected units had subsisted on rations similar to those described in Outbreak II, and these two periods of inadequacy should be regarded together. In those who contracted dysentery the resulting clinical picture varied according to any antecedent degree of malnutrition which was present. The error in the case of "K rations" lay in the assumption that men could subsist on them over an indefinite period without the additional and vital factor of variety.

The purely dysenteric cases responded quickly and satisfactorily to sulphaguanidine. In those with additional symptoms of glossitis, anaemia, etc., pale stools persisted after the course of sulphaguanidine, ordinary dietary additions were not tolerated and the institution of a sprue regime was necessary.

### 2 TWO CASES SEEN FOLLOWING PARTIAL TREATMENT

Brief details of the following cases are appended and will be referred to in the discussion. They are of especial interest in that they were seen several

months after treatment which was partial in that it was restricted to sprue dieting alone without parenteral liver therapy.

(1) *A Sergeant (R.A.F.)* who was one of the first to suffer in Outbreak 1 and before its significance was realized, was treated for enteritis. Whilst on leave symptoms returned, he was admitted to hospital and there treated for sprue by graded diets and oral liver extract. He was returned to his unit after 3 months. Intervened some 3 months later he was performing his duties but stated that since his return he had suffered from bouts of diarrhoea and that these appeared to have followed fatty meals. One meal of tinned bacon and fried chips had resulted in twenty loose stools. By recently restricting fats to a minimum he had kept tolerably well. He stated that he habitually passed semi-solid, large, pale stool each morning and a similar though looser stool later in the day. He remained about 2 stones (28 lb.) below his normal weight.

(2) *Lieut C.* This officer was seen in April, 1945 at hospital in Britain where he appeared for Medical Board following sick leave. Two months previously he had been invalided home from India as case of sprue. He was one of the cases whose symptoms had followed subsistence on R. Rations (for 10 weeks) and dysentery. Following treatment for the latter symptoms persisted and he was transferred to another hospital and diagnosed as sprue; treatment consisted of diets and oral liver extract. At this time stool analysis showed total faecal fat of 47 per cent. He improved, but having lost 2 stones in weight was invalided to Britain and admitted that during the months of his sick leave he had eaten ordinary diet to an attempt to gain weight. The tongue was red, and glazed in the centre; gastric analysis was within normal limits. R.B.C. count was 3,800,000 with colour-index of 1.1. He was regularly passing two, and sometimes three stools a day; the morning stool was sprue-like in appearance—pale, pulsatious and foul smelling—and later stools similar but small in amount. Stool analysis showed total faecal fat 14 per cent., of which 76 per cent. was split. Distension after meals was troublesome and his weight was still 2 stones below normal. He was admitted to hospital, and after 1 month sprue dieting and injections of whole liver he had gained 10 lb. in weight and the total faecal fat had decreased to 28 per cent.—a slow response as compared with similar cases treated in the early stage of the disease.

## DISCUSSION

There has been much difference of opinion as to whether the condition described was true sprue. Reference has been made to the disease described by NAPIER, CHAUDHURI and RAI CHAUDHURI, and COOK, noted chiefly among Indians, but named by the former *para sprue* and considered by these authors to be an entity distinct from sprue. If the observations recorded here are to have any value it must, therefore, be established beyond reasonable doubt that the disease under consideration was true sprue.

Diagnosis is difficult when at the outset it has to be admitted that we do not yet know what sprue is. The familiar biochemical, haematological and radiological tests employed for faecal fat, glucose-tolerance, anaemia, etc., have little or no specific value as they furnish no evidence of the initial pathological or biochemical lesion which initiated the process. They may provide evidence of intestinal malabsorption but this cannot be said to establish diagnosis of sprue since we are not by any means sure of what we are trying to establish. It would appear irrational, therefore, to use sprue as a diagnostic yardstick and to employ such terms as *para-sprue*, *pre-sprue*, *non-tropical sprue*, etc. until the standard of measurement has itself been accurately defined. The diagnosis of sprue is unhesitatingly used when the combination of clinical features and the results of laboratory tests (and usually a pronounced degree of abnormality is

insisted upon) together conform to a traditional pattern—that of the fully established clinical picture formerly presented by the European sufferer domiciled in the East. But it is fair to say that in cases which have not reached that degree of development traditionally associated with “typical sprue” the diagnosis is still bounded by the conception of the disease in the mind of the diagnostician. Developmental phases of sprue must inevitably exist and not the least important of the observations made in these three outbreaks was the existence of varying stages of development from mild prodromata to the severe picture of sprue itself.

The observations recorded will, therefore, be discussed in the following order (1) Clinical features, (2) Therapeutic response, (3) Aetiological factors

### (1) CLINICAL FEATURES

These comprised (in cases all of whom were suffering from a first attack) prodromata of lethargy, weakness, anorexia and gastro-intestinal upsets, later, diarrhoea, particularly matutinal, and abdominal distension, glossitis, dysphagia, and, in a few, angular stomatitis and apthous ulcers, loss of weight from moderate degrees to emaciation, anaemia, macrocytic in 40 per cent of the British and 62 per cent of the Indian cases haematologically examined, a tendency to achlorhydria and hypochlorhydria, flat curves in two and a low curve in one of the four cases examined for glucose tolerance, an absence of frank tetany but frequently occurring muscular cramps which may have been associated with hypocalcaemia, and signs of a malabsorptive vitamin B<sub>1</sub> deficiency in a small percentage.

Two individual features—faecal fat values and the tendency to watery stools—call for detailed discussion since many observers consider these to be at variance with a sprue diagnosis. The lack of uniformity of opinion in the interpretation of faecal fat analysis is well known. STANNUS has drawn attention to the numerous physiological factors involved in fat metabolism which must inevitably affect the amount and type of total and split fat excreted and which no analysis yet evolved takes into account, he tritely observes, “When the lower limit for normal faecal fat is given as 10 per cent and the upper limit as two and a half times that amount, it is obvious that the figures we obtain in sprue in the ordinary way can be but approximations.” Nevertheless, various authors place the “sprue-level” of total faecal fat at figures varying from 25 to 50 per cent and even higher, others attach more importance to the amounts of neutral and split fat. In attempting to reach a conclusion in a series of cases in an early stage of development, the important observation would appear to be whether there is consistent evidence of *any* abnormal excretion of fat (and carbohydrate) irrespective of arbitrary “sprue-levels” to which advanced cases may eventually attain. We know that in sprue an excessive excretion of fat through failure to absorb is an essential feature, and that the faecal fat should be fully split since there is no failure of pancreatic function. Accordingly the following interpretation is based, not on “sprue-levels” but on the accepted

belief that stools which contain more than a quarter of their dried weight of fat are abnormal.

In the above three outbreaks the stools were mainly pale, watery fermenting and contained an excess of undigested carbohydrate material stools of Indian cases were more watery than those of British cases which tended to a more pasty or porridgy consistence. The majority of all stools were greasy to the naked eye and microscopically showed an excess of fat globules and fatty acid crystals. Where analysis was possible there was found in the majority an amount of total and split faecal fat in excess of that which is generally stated to be normal. These features may be said to afford true evidence of an abnormal fat metabolism and excretion at an early stage in the disease. To any objection that the faecal fat percentages as a whole were not as high as those recorded in established sprue, attention is directed to the findings in the following (a) Case 7 Outbreak I total faecal fat 40 per cent. of which 90 per cent. was split, following an intake of only 80 grammes of fat daily (b) Gnr B., Outbreak II total faecal fat 48 per cent., neutral fat 2.5 per cent. fatty acids 17.5 per cent., soaps 26 per cent. (c) Lieut. C.—Miscellaneous cases total faecal fat 42 per cent., of which 78 per cent. was split (d) severe cases found in a Calcutta hospital to have total faecal fat of over 50 per cent. with excessive splitting. These analyses are consistent with those found in sprue and it is not proposed to define differently the other cases with lower (though still abnormal) faecal fat readings, but with identical clinical features, who were affected in the same areas and units at the same time and exposed to the same conditions.

Pale, watery diarrhoea is also not at variance with a sprue diagnosis. Several other authors have described this watery stool as occurring in certain phases of sprue, notably FAIRLEY (1936) and MANSION BAHR (1940). The latter describes the alternative type of stool as "evanescent, watery pale and fermenting with undigested food and, as a rule, an abnormally large amount of oil and fatty acids." COOK (1944) refers to the watery diarrhoea in his cases as justifying the isolation of the disease "as a syndrome allied to sprue, but he is careful to note, "I have, however seen stools exactly like those of classical sprue, pale, frothy hunky stools full of fat and fatty acids." Several factors came to light during the study of the above cases which go far towards explaining this variant of watery diarrhoea without invoking a different aetiology viz (1) COOK mentions excess of carbohydrate in Gujerat diets as a possible factor an opinion with which I agree a preponderance of carbohydrate (over 70 per cent.) was noted in the Indian troops diets and would tend towards the production of an irritant fermentative stool with an excess of undigested debris (2) the daily intake of fat was higher absolutely and relatively in British than in Indian troops, and the stools of the former were noted to be more pasty and porridgy than in the latter (3) lack of available minerals, especially calcium, was probably an important factor the daily intake of calcium was not inadequate but after days or weeks of diarrhoea and diminishing appetite it is probable that ensuing intestinal hurry permitted very little of the ingested calcium to





that a combination of clinical features of such an extreme severity appertains only to the advanced sprue sufferer. The mild and moderately affected cases in the three outbreaks conformed to NAPIER's description of para-sprue and these responded more quickly to treatment. But it is equally true that men from the same units (British and Indian) and affected at the same time as the former who were untreated, or partially treated, or who were obliged to continue the same dietary regime, eventually developed features indistinguishable from true sprue. As an example, Case 7 (Outbreak 1) was a gunner in a battery in which the majority suffered from mild symptoms only—he himself eventually exhibited a red, peeled tongue, macrocytic anaemia with R.B.C. count of 2,100,000 and C.I. of 1.3, a flat glucose-tolerance curve, gastric achlorhydria, a stool analysis showing total faecal fat of 40 per cent., of which 90 per cent. was split, peripheral neural signs of a vitamin B<sub>1</sub> deficiency and a loss of 49 lb. in weight. Whilst one might reasonably differentiate the mild and severe cases by such terms as early larval, or even acute sprue, it is irrational to use the term para-sprue if by that is implied an entity distinct from sprue with a different aetiology and pathogeny. Recognizable phases in development from mild forms to the severe hospitalized cases have been described previously. For the reasons given it is irrational to invoke a different diagnosis to explain clinical variations in a disease in which the fundamental essentials were identical and in which such variations could reasonably be attributed to degrees of development and secondary factors. Dissimilar in appearance as these earlier and later phases may be, they nevertheless should be regarded as stages in development of tropical sprue.

## (2) THERAPEUTIC RESPONSE.

The response of the cases to parenteral and oral crude liver extract, oral vitamin B<sub>1</sub>-complex preparations and high protein, low fat, low carbohydrate diets was precisely identical with that obtained in sprue denoting, by early disappearance of symptoms and restoration of normal intestinal function, that the pathogenic mechanism thus capable of correction was the same as in sprue. Whole liver extract was the "sheet-anchor" in treatment and the heightened response of those who were given the extract parenterally was such as to indicate the existence of a deficiency of certain components in crude liver—presumably fractions of the vitamin B<sub>1</sub> complex. Therapeutic response may therefore be said to have supported the contention that the syndrome was sprue, and to have indicated its deficiency nature.

## (3) AETIOLOGICAL FACTORS.

It is believed that there was a primary causal factor and several secondary or contributory factors. The primary factor is considered to have been a deficiency of vitamin B<sub>1</sub>-complex components due to prolonged dietary inadequacy. Support for the deficiency nature of the disease lies in the following findings.

1. The syndrome described by NAPIER, CHAUDHURI and RAI CHAUDHURI, and COOK

is believed for the reasons given to be a phase of sprue, these authors all stress a dietetic deficiency of vitamin B<sub>2</sub> complex in its production

2 Many individual features recorded such as glossitis, angular stomatitis and macrocytic anaemia are prominent in conditions known to be associated with deficiencies of components of this complex, *e.g.*, ariboflavinosis, pellagra, tropical macrocytic anaemia

3 Subnormal urine excretion levels of one component—nicotinic acid—were found, pronounced in severe cases, but also present in mild cases in which no loss from diarrhoea had occurred

4 Response to treatment in which parenteral crude liver and orally administered vitamin B<sub>2</sub> complex preparations were the sheet-anchor was pronounced and at times dramatic

Evidence that the deficiency was primarily dietetic is to be found in a study of the ration-issues to both British and Indian troops in the second and third outbreaks in which there had been inadequacy of riboflavin and nicotinic acid over a period of several months. This observation alone does not necessarily indicate that such a diet would be productive of sprue, but the close relationship in nature of components of the vitamin B<sub>2</sub> complex strongly suggests that when there is a deficiency of two such prominent fractions there is probably a deficiency of the other fractions also. Whatever arguments may be advanced against such a supposition one cannot evade the implication of the observation that in Outbreak II no cases of the disease were known to have occurred in the forward troops who were the recipients of a larger intake of fresh foodstuffs rich in vitamin B<sub>2</sub> complex components than was practicable at the Base where the outbreak occurred.

It should also be emphasized that in Outbreak II British and Indian troops suffered coincidentally from the same syndrome when their diets, though widely different in general composition, were found to be inadequate in the same vitamin fractions.

The deficiencies noted were not absolute and the amounts present are best described as minimal or border-line. Though deterioration in health and lack of vitality were to be expected, it is difficult to say whether any specific deficiency symptoms would have appeared under normal environmental conditions in previously healthy individuals subsisting on such rations. Secondary factors either accentuating any degree of deficiency already present, or actually precipitating a true deficiency state therefore assume aetiological importance. Previous discussion has included —

(a) Heightened metabolic demands from the rigours of arduous campaigning under tropical conditions

(b) Antecedent and concurrent diseases, with particular stress on malaria and dysentery, the former with its heightened metabolic demands and the anaemia to which the sufferers from repeated attacks is subject, and dysentery, with its acute deprivations from diarrhoeic loss and impaired absorption through rapidity of passage of the intestinal contents. Though malaria and dysentery were not shown to be essential forerunners of sprue, it is a fair assumption that sufferers from such diseases are rendered more susceptible to dietary deficiencies, the higher incidence of Indian, as compared with British cases, especially in Outbreak II, supports the contention

(c) Anorexia, with progressive reduction of food (and vitamin) intake after commencement of symptoms, due to vitamin deprivation itself, to general lack of dietetic variety, and in many British cases to a gastric upset from the ingestion of unwholesome fats

(d) Dietetic imbalance. This speculative subject will only be briefly alluded to here —

(1) In British cases in the first and second outbreaks there was available evidence of the ingestion of an excess of unwholesome fatty foods. Did the latter contribute to the pathology merely by inducing an irritant gastro-enteritis with diarrhoea and malabsorption, or did they additionally and specifically effect some state of metabolic imbalance? It is opportune at this juncture to refer to the theory advanced by STANOUT (1945) which is in turn based on the "fat-fatty acid of partition hypothesis" of FRAZER (1938-1940) and FRAZER and STEWART (1936). The essence of the theory is that the co-enzymes which catalyse the process of phosphorylation probably embody certain fractions of the vitamin B<sub>2</sub> complex, which are present in crude liver extract—that glycerol, glucose and certain fatty acids are dependent on phosphorylation for their absorption by the intestinal mucosa, as opposed to neutral fat and such carbohydrates as fructose which may be absorbed without phosphorylation—and that failure of this process through lack of these vitamin co-enzymes leads to an accumulation first of unabsorbed fatty acids and glucose, and secondarily to non-absorption of neutral fat and other carbohydrates—calcium deprivation follows through fixation with excess fatty acids and the formation of calcium soaps, whilst phosphorus depletion results from defective phospholipid formation through failure of phosphorylation. If I understand this hypothesis correctly then the requirements of the operative components of the vitamin B<sub>2</sub> complex are relative to the amount and nature of the fatty acids (and carbohydrates) consumed by the individual and that a high fat diet, or one containing those particular fatty acids which have high phosphorylation requirement will demand proportionately higher "cover" of the operative vitamin fractions for their eventual absorption.

There is no doubt that the peculiar fatty ingredients of the diets in British cases caused gastro-enteritis with resulting anorexia, nausea, vomiting (in some cases) and diarrhoea, and thereby contributed to the deficiency syndrome by this mechanism alone: the enteritis in Outbreak I was so acute as to suggest that it was the dominant precipitating factor. But it is also difficult to escape the conviction that the metabolic demands of the high fatty acid content of the diet were such as could not be met by the inadequate "cover" of vitamin B<sub>2</sub> complex. Whether this was relative to the total fats per se or to certain individual fatty acids, is not known. Facilities were not available for an analysis either of the numerous fatty acids ingested in the rations, or of the fatty acids present in the stools. Such an investigation may eventually show that certain fats require optimal amounts of vitamin B<sub>2</sub> complex fractions for their phosphorylation. Whilst it is the broad aetiology of sprue rather than its pathology which is the subject of these notes, attention is directed to the emphasis given by BRACH (1936) and STANOUT to the possibility that vitamin B<sub>2</sub> (pyridoxin) may be actively implicated in the absorption of unsaturated fatty acids by the intestinal mucosa. Riboflavin and nicotinic acid are also believed to be phosphate "carriers," and may be concerned in the phosphorylation of glucose and glycerol on their absorption from the intestine. The low amounts of riboflavin and nicotinic acid in the rations in the second and third outbreaks have been discussed. The amounts of pyridoxin in the various tinned and other foods are not known, but only small quantities of the commonly known sources of pyridoxin were issued, and foods with high values—corn-oil, wheatgerm, peanut and linseed oils, egg yolk, liver etc.—were absent from these diets.

(2) In Indian troops not only was the daily caloric value of the diet high, but there was evidence of a protein-carbohydrate imbalance. Carbohydrate constituted over 70 per cent. of the total intake. STANOUT's theory includes lack of phosphorylation of carbohydrates (glucose) in sprue through inadequate vitamin B<sub>2</sub> complex components. SYRINGER (1941) showed that in persons subsisting predominantly on carbohydrate diet, features attributable to riboflavin and nicotinic acid deficiencies were apt to arise unless large cover of vitamin B<sub>2</sub> complex was provided.

The secondary or contributory aetiological factors enumerated above were individually and collectively so pronounced as to have demanded, on general nutritional principles, heightened vitamin intake rather than minimal one.

#### *Vitamin B<sub>12</sub> biosynthesis.*

The following note is put forward purely hypothetically and concerns the biosynthesis of vitamins in the bowel. It has been shown in recent years that the intestinal flora of

some animals can synthesize certain vitamins, the rat has been shown capable of synthesizing vitamins C, K, E, thiamin, riboflavin, pyridoxin, nicotinic acid, biotin, pantothenic acid, folic acid and inositol. NAJJAR and HOLT (1943) have shown that thiamin and riboflavin are synthesized in the human bowel, and ELLINGER *et al.* (1944, 1945) have recorded a similar biosynthesis of nicotinamide to the extent of approximately 80 per cent of estimated human requirement. What is still in doubt is the extent to which vitamins so produced are utilized by the human host. The knowledge that cats, rats and dogs synthesize vitamin C and do not suffer from scurvy is important. Does man also rely on a dual source of supply for certain of his vitamins—those in his diet and those synthesized by his own intestinal flora? Whilst the intrusion of such an element in pathogeny is as yet entirely speculative, the possibility has to be envisaged that certain intestinal disorders may produce deficiency syndromes by depriving the individual of his synthesized vitamins. Applied, for example, to the outbreaks of sprue recorded above, did the intestinal derangement produced by the ingestion of unwholesome fats create in the bowel a condition inimicable to the synthesis of essential components of the vitamin B<sub>2</sub> complex, thus rendering the small amounts taken orally totally insignificant for the individual's needs? Against the possibility of such a mechanism in sprue is the meagre evidence of the results of treatment of the cases admitted to hospital with dysentery, of whom a percentage had sprue features in addition following subsistence on "K Rations" described earlier. For the reasons previously given, it is believed that all these patients, irrespective of the presence of sprue symptoms, were border-line cases of vitamin B<sub>2</sub> complex deficiency. All of them received a full course of sulphaguanidine, but it is to be noted that in no case were sprue symptoms aggravated, nor did any dysenteric case without sprue symptoms develop them. An event which, theoretically, ought to have occurred following depletion of intestinal bacteria and synthesized vitamins had the above mechanism been operative.

Added speculation would be idle, but further research in this field of bacterial vitamin synthesis may reveal much that is not at present understood in the production of, and natural protection against, deficiency syndromes.

## CONCLUSION

The above discussion may be summarized in the statement that the syndrome described was tropical sprue in various stages of development, that the therapeutic response supported such a diagnosis, and that the primary aetiological factor in its production was believed to have been a dietary deficiency of certain components of the vitamin B<sub>2</sub> complex.

In treatment, immediate and thorough therapy, even in the early, mild cases, with large doses of parenteral crude liver extract is strongly urged. Not only is the response more rapid and satisfactory (as compared with oral administration) and permitting of a quicker "stepping-up" of nourishment, but there is accumulating evidence that the more speedy restoration of the patient's vitamin balance—and probably reserves—is to his ultimate advantage in decreasing the likelihood of relapse. Mere amelioration should not be mistaken for complete restoration to normal function.

It will be obvious that in the writer's opinion predominant importance attaches to the recognition of the basic aetiological factor in production of the syndrome. Provided this is fully recognized, and the other factors correctly but subordinately placed in their precipitating, contributory and variant-producing roles, the label attached to the syndrome is of secondary importance only. Without such recognition, prevention and rational therapy must inevitably be retarded. The name "sprue" is of historic interest and will almost certainly be retained though a term embodying the precise vitamin fraction, or fractions,

implicated—when these have been finally elucidated—would be preferable. (Certain American authorities now express B avitaminosis in terms of first, second, third, etc., degrees of clinical involvement, reserving for the sixth degree—and in parentheses—the term beriberi.) The writer in India, used the term "Malnutrition Vitamin B complex deficiency" though not in accordance with the War Office *Nomenclature of Diseases* it appeared preferable to the term "sprue" in that it served for all degrees of severity and, in the event of cases being transferred to other hospitals, was explanatory in terms of aetiology and the therapy indicated.

Objection was occasionally voiced in India that the epidemic nature of the disease was itself a cogent argument against a sprue diagnosis. Nevertheless, before the war the existence of "sprue areas," and sprue bungalows was recognized. It should be borne in mind that during the past 5 years large tracts of India and Burma have been inhabited by tens of thousands of British personnel where previously a mere handful existed. Factors which formerly would have affected a few only have recently had the opportunity of affecting thousands. Historically unique an epidemic of sprue among British personnel may be, but the site and the conditions of the military campaign were also unique. When all the facts are reviewed these outbreaks are no more bewildering than were the first reports of the epidemics of beriberi which afflicted British troops in Mesopotamia during the war of 1914-18.

The syndrome described may be a disabling disease at any time that it may result in a serious drainage of man-power during a campaign is reflected in the fact that these notes refer to approximately 3 000 cases, mild, moderate and severe, which came within the orbit of the writer during service in two areas only.

The above observations support the view that tropical sprue rightly belongs to the diseases of malnutrition and indicate a means to its prevention.

## SUMMARY

1. Three outbreaks of sprue in epidemic proportions are described. The affected personnel were British and Indian troops in Burma Campaign, 1943-44. Including all degrees of severity approximately 3 000 were affected.

2. The majority suffered from a mild or moderate form, conforming to that recently described as para-sprue but as cases from the same units and areas developed severe symptoms clinically indistinguishable from true sprue the disease is believed to have been, aetologically and pathogenically the same throughout, i.e., sprue. There was no discernible fundamental difference between British and Indian cases.

3. The clinical features in general were identical with those traditionally associated with sprue. The noteworthy variants were the watery more frequent and less bulky stools, and their lower fat content as compared with "typical" sprue. Factors responsible for these variations are considered to have included the duration of the disease, the amount and nature of fats (fatty acids) and

carbohydrates ingested, rapidity of passage of the intestinal contents, and lack of available minerals, especially calcium. Ample soap-formation yields more solid stools, and the lesser-irritant calcium "sprue-stool" is probably the expression of megacolon rather than of sprue *per se*, and should be regarded as a later (though not inevitable) development rather than as an essential diagnostic feature in the early phases of sprue.

4 Varying stages in the development of sprue were recognizable from prodromata only, to mild, moderate and severe forms. Not all cases showed the same combination of features or severity of individual symptoms. The commonest features, in average order of development, were lethargy, muscular weakness, anorexia, flatulent dyspepsia, pale diarrhoea, glossitis, anaemia and loss of weight.

Even in the early phases an abnormal fat excretion was evidenced in the pale, greasy nature of the stools, excess of fat globules and fatty-acid crystals microscopically, and a total faecal fat of over 25 per cent with excessive splitting in the majority of those analysed. The stools were additionally fermentative and contained excess of carbohydrate debris. Such stools are considered to be as truly indicative of an abnormal fat (and carbohydrate) metabolism and excretion as those of advanced sprue in which extremely high faecal fat percentages may be found.

5 Aetiologically, the primary causal factor was considered to have been a prolonged dietary inadequacy of one or more of the components of vitamin B<sub>2</sub> complex. There was a calculable deficiency of riboflavin and nicotinic acid in the rations issued which were also poor in pyridoxin values. This evidence is considered to be presumptive of a general deficiency of vitamin B<sub>2</sub> complex components which are closely associated in nature. Further support for the deficiency-nature of the disease is found in the known association of certain individual clinical features with deficiencies of specific fractions of the complex, the reported association of a deficiency of vitamin B<sub>2</sub> complex foods with parasprue, the excretion of subnormal amounts of nicotinic acid, and the therapeutic response to parenteral and oral crude liver extract and other vitamin B<sub>2</sub> complex preparations.

In addition, troops subsisting on rations richer in fresh foods containing this complex did not contract the disease. British and Indian troops suffered considerably from the syndrome in the same area when their diets, though widely different in general composition, had a common inadequacy of vitamin B<sub>2</sub> complex.

6 Secondary aetiological factors including the increased metabolic demands of a rigorous campaign—malaria, dysentery, antecedent malnutrition and anaemia, progressive deterioration of appetite and, hypothetically, dietetic imbalances of which the high carbohydrate intake in Indian cases and excess of (unwholesome) fats in British cases—are stressed. The possible dual role in pathogeny of these excesses is discussed both in terms of gastro-intestinal irritation (as applied to

fats) with malabsorption and diarrhoea, and also with reference to STANUŠ's hypothesis that sprue is the expression of a failure of phosphorylation of glucose and fatty acids through lack of co-enzymes which catalyse the process, these including certain and as yet unspecified fractions of the vitamin B<sub>2</sub> complex. The vitamin B<sub>2</sub> complex fatty acid/carbohydrate ratio in the cases described lends support to the theory of such a mechanism.

7. There was no evidence that the development of sprue was dependent on an infective agent, antecedent intestinal disease or "inherent metabolic errors."

8. Therapeutic response to modern sprue therapy was pronounced and supported the diagnosis. The heightened response to crude liver extract given parenterally supports the view that sprue is associated with a deficiency of one or more substances present in liver presumably fractions of the vitamin B<sub>2</sub> complex.

9. That these outbreaks occurred in epidemic proportions is not a cogent argument against a sprue diagnosis in view of the factors involved.

10. The observations made support the view that tropical sprue is a disease of malnutrition and indicate means to its prevention.

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GROWTH OF PROTOZOA IN TISSUE CULTURE  
IV *PLASMODIUM LOPHURAE*, EXOERYTHROCYTIC FORMS,  
IN VIVO AND IN VITRO

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The present paper describes the exoerythrocytic forms of *Plasmodium lophurae* as seen in turkeys, and their growth in tissue culture by the technique described for *P. gallinaceum* and other parasites in previous papers of this series (HAWKING, 1945, 1946). *P. lophurae* was isolated from a Borneo pheasant by COCCESHALL (1938), and it has been used extensively for chemotherapeutic investigations in America and in this country as an infection in ducks or chicks. No exoerythrocytic forms had been reported in canaries, ducks or chickens. In 1944, however, we learnt that PORTER and LAIRD had demonstrated these stages by inoculating young turkeys with sporozoites of *P. lophurae*. The present work was undertaken to confirm their work and to study the appearance of these forms in tissue culture.

EXPERIMENTS

*Aedes albopictus* mosquitoes were fed on a chick infected with *P. lophurae*, and approximately 50 per cent of the mosquitoes developed infection in the glands. The mosquitoes were killed, ground in saline, centrifuged and suspended in equal parts of Ringer's solution and chick serum. The suspension was inoculated intravenously into two 14-day-old turkey chicks, the birds receiving the equivalent of thirty and fifteen infected mosquitoes respectively. Blood smears taken on the 6th day after infection showed

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1 per cent. of the red cells to be parasitized and the birds looked quite healthy but they died during the night of the 6th to 7th day. Blood smears taken postmortem showed 7 per cent. of the red cells to be parasitized. Smears of the brain and sections of the organs all showed the presence of numerous tissue forms (The tissues were fixed in Zenker formal and the sections were stained with Giemsa or with haematoxylin-eosin-azure II, Buxley and Buxley 1935.)

The experiment was repeated on further turkey chick which received fifty infected mosquitoes in 0.8 ml. of equal parts of Rieger's solution and chick serum. The bird was killed on the 5th day after infection when blood smears showed 0.8 per cent. of the red cells to be parasitized. Very few exoerythrocytic forms were found in the organs. Judging by the previous experiment, large increase must take place in these forms between the 5th and 7th days after infection.

The spleen was removed aseptically and planted out in Carrel flasks using the method described by H. Wicker (1945). The plasma was obtained from fowl and the fluid phase consisted of Tyrode solution containing 20 per cent. turkey serum, 8 per cent. chick embryo extract, three units per c.c. of penicillin and 0.05 per cent. phenol red. Growth of cells occurred in nine out of fourteen flasks set up. Some explants of heart muscle and liver were set up but they failed to grow. Some explants of bone marrow gave profuse growth of cells like fibroblasts but no parasites were seen in them even when the growth of cells was good.

The spleen cultures were maintained for 13 days at 37° C. and were sampled during this period on the 6th, 8th, 9th and 13th day. Sampling was carried out by removing a slip from one or more of the flasks. The slip and its adherent culture were fixed in Schaudinn's solution, stained overnight with alloxalin Giemsa (Giemsa 2.5 c.c., methyl alcohol 5 c.c., 0.5 per cent. sodium carbonate 10 drops, water 100 c.c.) differentiated by passing through dilute acetic acid, dehydrated with acetone and xylol, and mounted in neutral mountant.

Parasites were seen in some of the samples taken on each day and all the flasks which showed growth of spleen cells also showed growth of parasites on one or more of the slips set up in them. On some of the slips many parasites were seen, usually localized in patches as had been the case with *P. gallinaceum*.

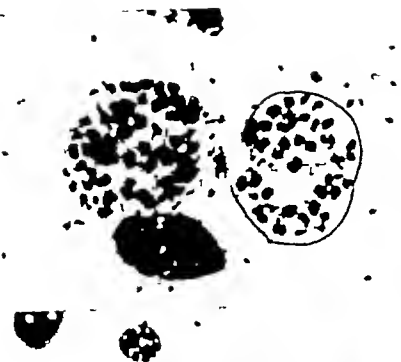
### MORPHOLOGY OF THE EXOERYTHROCYTIC FORMS IN VIVO

In the first two turkey chicks described above great numbers of exoerythrocytic parasites were found in smears and sections. They were very numerous in the capillaries of the brain, heart, glomeruli of the kidney and cortex

#### CAPIONS: FIGS. 1 TO 8.

- FIG. 1.—Circular exoerythrocytic schizonts of *P. lophurae* from smear of brain. (One schizont has been outlined in ink.)
- FIG. 2.—Elongated schizonts in capillary of the brain; dark purple chromatin and pale blue cytoplasm. Smear.
- FIG. 3.—Similar schizonts with dark blue cytoplasm and small pale pieces of chromatin. Smear.
- FIG. 4.—Elongated schizont in capillary of brain from turkey used for tissue cultures. Paraffin section.
- FIG. 5.—Schizont from lung of turkey used for tissue culture. There is clear area in the centre of the parasite. Paraffin section.
- FIG. 6.—Exoerythrocytic form in capillary of brain. Celloidin section.
- FIG. 7.—Large schizonts in capillary of the brain, cut in transversely to show the arrangement of involution layers. Celloidin section.
- FIG. 8.—Macro-megakaryotes in section of heart. Celloidin section.

Magnification Figs. 1-8.  $\times 1250$ .



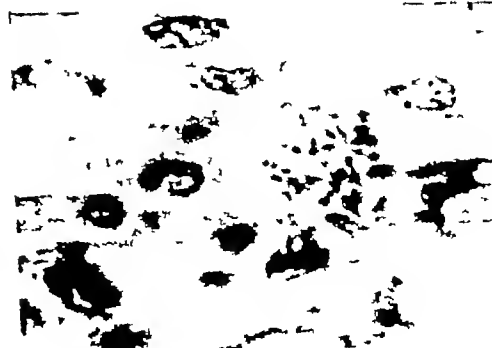
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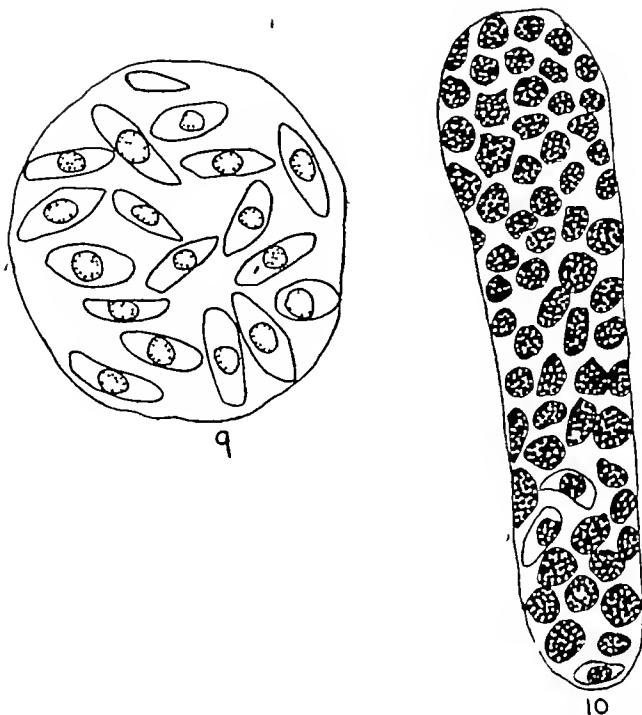


FIG 9 —Macro-merozoites in capillary of heart, drawn from celloidin section  $\times 1,250$   
 FIG 10 —Schizont with micro-merozoites in capillary of heart, drawn from celloidin section

Figs 9 and 10 drawn by F H

of the adrenals, they were few to moderate in the spleen, lung, capillaries of the renal tubules and sympathetic ganglia, they were not seen during a moderately long search in preparations from the liver and bone marrow, although presumably a few must have been present there. In the chick used for the tissue culture experiment, parasites were rather scanty, they were seen in the brain, kidneys (capillaries of the tubules), lungs and spleen but not in the heart or marrow.

#### SMEARS

In smears, the forms found were mostly large schizonts with ten or more pieces of chromatin. Forms found in smears of spleen were circular and so were a few of those in smears of the brain (Fig 1), while most of those found in smears of brain were sausage shaped (Figs 2 and 3). In general the forms closely resembled those of *P. gallinaceum* as described by JAMES and TATE (1938), but division into cytomeres (as shown in their Fig 10) was not seen. Some of the forms had abundant chromatin and a fair amount of cytoplasm.

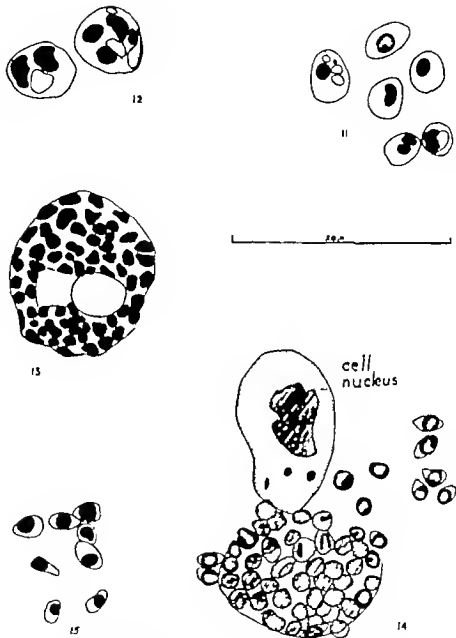


FIG. 11.—Group of small parasites with one or two pieces of chromatin, from tissue cultures. Two parasites contain vacuoles.

FIG. 12.—Larger forms with two or five pieces of chromatin, from tissue cultures.

FIG. 13.—Large schizont, showing clear areas.

FIG. 14.—Schizont breaking up and liberating micro-merozoites.

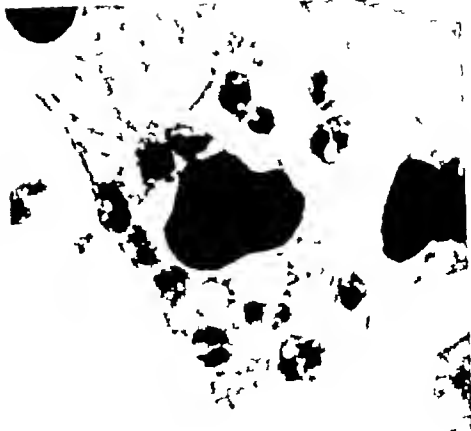
FIG. 15.—Group of merozoites.

Magnification of Figs. 9-14 shown by scale (Fig. 11)

16



17



18



19



FIG 16 —Group of small parasites in a cell in tissue culture  
 FIGS 17-18 —Larger parasites with 2-7 pieces of chromatin  
 FIG 19 —Large immature schizont

20



22



FIG. 20 — Large schizont showing clear area.

FIG. 21 — Large mature schizont, in which microzoites are being formed.

FIG. 22 — Schizont liberating micro-metazoa.

FIG. 23 — Four or more large schizonts overlying group of cells.

As in Figs. 21 and 23 the parasites lie in different optical planes, the photographs have been superimposed.

staining pale blue (Figs 1 and 2) while others had a great amount of cytoplasm staining dark blue and only small pieces of chromatin (Fig 3). But it was not clear that these were two fundamentally different types of schizont, they may have been different stages of the same parasite, or the difference in appearance may have been due to different degrees of flattening. Fully formed merozoites were not encountered while examining the smears.

#### SECTIONS

In the sections, only the more mature stages of the parasites could be distinguished with any certainty. The appearances in general (Figs 4 to 10) resembled those of *P. gallinaceum* as described by JAMES and TATE (1938). In some forms (Fig 5) the rounded pieces of chromatin were arranged round masses of cytoplasm (JAMES and TATE, Fig 13) and in others (Fig 7), the schizonts were arranged in involuted layers inside a capillary (JAMES and TATE, Fig 18). Forms containing many "macro-merozoites" arranged irregularly inside a capsule, as depicted by HUFF and COULSTON, 1944 (their Fig 64A) were fairly common and such forms were more prominent in the sections than they were in the tissue cultures (Figs 8 and 9). Forms consisting of "micro-merozoites" (HUFF and COULSTON, their Fig 64B) were seen only occasionally (Fig 10). Some of the capillaries contained collections of small parasites which looked as though they had only recently been liberated.

#### MORPHOLOGY OF THE EXOERYTHROCYTIC FORMS IN TISSUE CULTURE

Many different forms of plasmodia were seen in the cultures and the main types will be described in the probable order of their development, but since one particular parasite cannot yet be followed throughout its whole life history, the cycle of development can only be presumed at present not actually proved.

##### 1. FORMS WITH ONE PIECE OF CHROMATIN (Figs 11 and 16)

These were usually rounded in shape and varied in diameter from about 2.3 to 4.5  $\mu$ . The chromatin was irregular in shape and sometimes angular, usually it measured 1.5 to 3  $\mu$  across. (Note, all these dimensions depend greatly on the degree of flattening of the parasites.) The chromatin stained crimson more or less darkly, sometimes the outer part stained more deeply than the centre. In some of the parasites the chromatin was elongated or shaped like a figure of eight as though it were in process of division. The cytoplasm stained pale blue, many parasites contained one or more small vacuoles which may represent a degenerative change. In many of the parasites also a small granule, which stained dark red or brown, was present.



near the chromatin. Presumably these forms developed from the meronts after they entered a new host cell. They closely resembled the similar stages of *P. gallinaceum* or *P. relictum*.

2. SMALL SCHIZONTS WITH TWO TO TWENTY PIECES OF CHROMATIN.  
(Figs 12, 17, 18, 19).

These were similar in their general features to the forms with one piece of chromatin from which they are presumably derived but several pieces of chromatin were present. The cytoplasm stained bright blue and was relatively abundant compared with the chromatin, especially in the smaller forms. Vacuoles and granules may be present in the smaller forms.

3. LARGE SCHIZONTS WITH MORE THAN TWENTY PIECES OF CHROMATIN  
(Figs 13, 20, 23).

These were round or oval and measured up to 20 $\mu$  in diameter. They contained up to seventy or more pieces of chromatin. The chromatin masses stained dark purple and were rounded or slightly oval in shape. Except in the smaller forms the cytoplasm was scanty and difficult to distinguish. Usually it seemed to be concentrated as thin envelopes round the masses of chromatin. In some of these schizonts, one or more clear spaces might be discernible, as with *P. gallinaceum* (HAWKING 1945 Figs. 10 and 11), but they were seldom large or conspicuous, and the chromatin masses were not arranged round them in any very regular way (Figs. 13, 20). These forms were much less common or conspicuous than they were with *P. gallinaceum* and somewhat less common than with *P. relictum*. The relation of these forms to those described in 4 is not clear.

4. FORMS IN WHICH MEROZOITES ARE FORESHADOWED (Fig. 21).

These resemble the previous forms (3) in shape and size but the cytoplasmic envelope round some of the pieces of chromatin is sufficiently well marked to form pre-merozoites. The latter are usually egg-shaped with the chromatin at one end, but elongated forms with the chromatin in the middle were seen in some cases. No definite arrangement of the pre-merozoites was seen, except that those near the outside tended to be arranged circumferentially. The forms seen with *P. relictum* in which elongated merozoites were arranged radially were not seen in these cultures.

5. FORMS UNDERGOING SCHIZOGONY (Figs 14 and 22)

These forms were similar to those of the last paragraph but some of the outer part of the parasite had broken down liberating merozoites before and during this process.

## 6 FREE MEROZOITES (Fig 15)

Most of these consisted of a rounded mass of chromatin 1 to  $1.5\mu$  across sometimes the centre stained less dark than the periphery. The cytoplasm surrounding this was often arranged to one side, forming a round or egg-shaped mass  $1.5$  to  $2.5\mu$  long by  $1.5\mu$  wide. Presumably these were merozoites. Occasionally larger forms were also seen, in which the chromatin was about  $1.5\mu$  across while the cytoplasm was more abundant and measured up to  $4.5 \times 2.2\mu$ . These resembled macro merozoites. Apparently the merozoites entered adjacent cells and became the forms with one piece of chromatin described in 1. The merozoites did not seem to be distributed at all widely from the point at which they were liberated, and multiple infections of cells (up to forty-two in a single cell) were common. Similarly the distribution of the parasites in different parts of the culture was very uneven. In some parts, parasites were very numerous, in other adjacent parts they were rare or absent. In these features, the cultures resembled those of *P. gallinaceum* and *P. relictum*.

Degeneration of the host cell, due to the parasites in it was rarely seen although presumably it sometimes occurred. When liberation of the merozoites occurred the host cell was probably destroyed, but this was not certain. It is difficult to believe that forty-two parasites could complete their development into large schizonts inside a single cell. In Fig 23 a small group of cells is shown, which is overgrown by a mass of four or more schizonts so closely pressed together that the outlines of the individual parasites are almost indiscernible. In an adjacent part of the same culture there was a large blue mass (measuring about  $90\mu$  across), in which the outlines of degenerate macrophages and numerous parasites could be discerned with difficulty suggesting that the parasites had become so numerous as to cause degeneration of the cells and consequently of themselves. Similar appearances were seen in other cultures.

## DISCUSSION

In general features these tissue cultures of *P. lophurae* resembled those of *P. gallinaceum* and of *P. relictum* which have already been described. The chief differences from *P. gallinaceum* were that the large schizonts with clear spaces (presumed to be stages leading up to schizogony) were less conspicuous with *P. lophurae* than with *P. gallinaceum* but this difference was not enough to separate the two parasites and morphologically the exoerythrocytic forms of *P. lophurae* found in smears and sections were indistinguishable from those of *P. gallinaceum*. Compared with *P. relictum* *P. lophurae* showed no evidence of radial arrangement of the 'pre merozoites' inside the almost mature schizonts.

Two kinds of merozoites viz. macro and micro-merozoites arising from exoerythrocytic schizonts have been described by HUFF and COLESTON (1944)

and by MUDROW and REICHENOW (1944). The latter workers believe that the macro-merozoites parasitize tissue cells, while the micro-merozoites parasitize erythrocytes. Schizonts of *P. lophurae* corresponding to these two kinds of merozoites were seen in the sections as noted above but the characterization of these forms in tissue culture presents certain difficulties and will not be discussed here in detail. Merozoites were seen in many of the preparations and in great numbers. Most of them had only scanty cytoplasm and corresponded to micro-merozoites as described by the above workers similarly there were many mature schizonts containing numerous pieces of chromatin, and relatively little cytoplasm, i.e. schizonts developing into macro-merozoites. On the other hand, merozoites with abundant cytoplasm, i.e. macro-merozoites, were much less easy to identify they were difficult to distinguish with certainty from forms which had entered a new host cell and had begun to develop as young trophozoites. Similarly schizonts leading to macro-merozoites were also difficult to identify with certainty which is in contrast to the prominence of these forms in the sections. The long sausage-shaped parasites seen in the capillaries of the brain and heart were not reproduced in the tissue cultures.

The ready growth of the exoerythrocytic form of *P. lophurae* in tissue culture supports the generalization made in previous papers of this series that probably most or all malaria parasites which grow in macrophages or similar cells should be susceptible of cultivation by this technique.

### SUMMARY

1. In confirmation of the report by PORTER and LAIRD exoerythrocytic forms of *P. lophurae* have been demonstrated by inoculating young turkeys with sporozoites of this plasmodium.
2. These exoerythrocytic forms have been grown in tissue culture.
3. The morphology of these forms *in vivo* and in tissue cultures is described and illustrated. The forms closely resemble the exoerythrocytic forms of *P. gallinaceum*.

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## POLYMORPHISM IN *TRYPANOSOMA SIMIAE* AND THE MORPHOLOGY OF THE METACYCLIC FORMS

BY

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AND

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The blood of a horse infected with a polymorphic trypanosome was inoculated into a sheep, passaged to a second sheep, and boxes of clean, laboratory-bred *Glossina morsitans* were fed on the latter animal. Mr F L VANDERPLANK, of the Tsetse Research Department, Shinyanga, kindly sent us these boxes, from which we isolated a fly, QMI. This fly was fed on Monkey 711 on 16th May, 1945, and we transmitted the strain cyclically by *G. morsitans* in *Cercopithecus* monkeys until it was abandoned in November, 1945.

We had been trying to obtain a strain of *T. brucei*, but the trypanosomes in the first monkey's blood were so different from the usual *brucei* picture, and the morphology of the metacyclic trypanosomes found on slides probed on to by infected flies were so unusual, that we dissected a fly, and found trypanosomes only in the stomach and proboscis. A study of the literature confirmed the fact that the strain was really *T. simiae*, a conclusion supported by our failure to infect rats and guinea-pigs.

The duration of life of the nine infected monkeys, calculated from when the trypanosomes first appeared in the blood, varied from 4 to 6 days, with an average of 4.7 days, just long enough to secure a fly transmission. From many of the monkey hosts we took daily thin blood films from the time trypanosomes first appeared in the blood until the animal's death, and so we had ample material from which to study the morphology of the strain. We attempted to passage it by fly through goats to study the effect on the subsequent virulence to monkeys, but all the goats failed to be infected.

\* We have to thank the DIRECTOR OF MEDICAL SERVICES, Tanganyika Territory, for permission to publish this paper.

The trypanosomes present corresponded very closely in their general morphology to Hoar's description (Hoar, 1936a, 1936b), and consisted of the three types he mentioned, namely the classical *sinai* forms, long and stout with a well-developed undulating membrane, the slender *rhodaini*-like forms with an underdeveloped membrane and the *congolense*-like forms. The classical *sinai* form predominated in all our slides, the proportion of *rhodaini*-like forms fluctuated considerably from nearly zero to about 24 per cent., while the proportion of the *congolense*-like forms also varied though they were never numerous. This is in conformity with Hoar's findings.

An attempt was made to determine the sign of the electrical charge carried by the trypanosome, by the thin-film method described by us (Fairbairn and Culwick 1946), but the attraction or repulsion between the trypanosomes and the red blood cells was so slight that the charge could not be determined accurately. It is regretted that we did not put up a suspension in the glucose-saline solution of Brooks, Brown and Hoar (1936) to see whether the strain was positively or negatively charged or mixed. The trypanosomes were therefore measured without any distinction as to charge, and the results are recorded in the table below.

TABLE  
STATISTICAL ANALYSIS OF MEASUREMENTS OF THE THREE FORMS OF *T. sinai*.

	<i>Congolense</i> -like form.	<i>Rhodaini</i> -like form.	Long, stout form
Range of length	9-17 $\mu$	11-20 $\mu$	14-23 $\mu$
Mean length ( $\mu$ )	13.27	13.79	18.00
Standard error ( $\mu$ )	$\pm 0.43$	$\pm 0.14$	$\pm 0.21$
$S_1$	0.09	0.23	0.26
$\sigma S_1$	$\pm 0.31$	$\pm 0.23$	$\pm 0.24$
$S_2$	0.14	0.84	— 0.46
$\sigma S_2$	$\pm 0.41$	$\pm 0.80$	$\pm 0.47$

From the statistical analysis it will be seen that the lengths of each form had a frequency distribution which did not depart significantly from normality and *T. sinai* must therefore be considered as truly polymorphic. We found also that 24 per cent. of the long, stout forms were in a state of division but no case was observed of either of the other two forms dividing. The metacyclic trypanosomes which we found on slides probed on to by infected flies were too few to allow of quantitative analysis. They consisted, however of two types, one with and one without a flagellum, the lengths of those which were measured ranging from 10 to 12  $\mu$ . The figure shows camera-lucida drawings of typical forms.

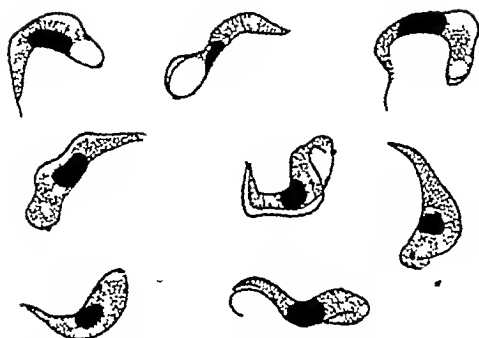


FIG.—Camera-lucida drawings of the metacyclic trypanosomes, of *T simiae* in *G morsitans*

### DISCUSSION

In a previous paper (FAIRBAIRN and CULWICK, 1946) we have shown that *T rhodesiense* exists in three forms, a short, an intermediate and a long one, each form occurring as a positively and a negatively charged variant, i.e. six types in all, each with a length frequency distribution which does not depart significantly from normality, that it is only the long form which divides by binary fission in the blood, and that the metacyclic trypanosomes of *T rhodesiense* exist in two forms, one with and one without a free flagellum. We have shown that syngamy occurs in *T rhodesiense*, and from a consideration of genetic factors we argued that the long blood form must be heterozygous, while the short and intermediate forms—and probably also the two metacyclic forms—must be homozygous.

We have already shown (loc cit) that syngamy occurs in *T simiae* also. As the typical long, stout form of *T simiae* is apparently the only one which divides by binary fission, it also must be heterozygous, while the other two forms must be homozygous.

In another attempt to procure strains of *T brucei*, we examined the blood of cattle at Chungai, Kondoa Irangi District, which had polymorphic trypanosomes, and again discovered *T simiae*. It would appear that this trypanosome is commoner than has been supposed in the past, and its polymorphism is apt to lead to a diagnosis of *T brucei*, or *T congolense* may be diagnosed if the short forms are present in any numbers. It is therefore essential to keep the possibility of *T simiae* in mind in order to establish the correct diagnosis in horses and cattle, as these animals may be reservoirs causing outbreaks in domestic pigs. In *T congolense* the typical forms divide by binary fission, but the short *congolense*-like forms of *T simiae* do not, so far as we have observed, and this should help in the separation of these two species. What the reaction of horses and cattle, infected with *T simiae*, to drug treatment is—remains to be established.

## SUMMARY

1 HOARE'S findings regarding the morphology of *T. simiae* are confirmed.

2 It is shown that *T. simiae* is trimorphic, and that the length-distributions of the three forms do not depart significantly from normality

3 Metacyclic forms of *T. simiae* are figured.

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## INFECTION OF MAN WITH *LEPTOSPIRA BOVIS* IN PALESTINE

BY

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Outbreaks of jaundice are well known in the Middle East, and special attention has been called to them in Palestine during World War II by CAMERON, LEFKOWITZ, BTESH, KLIGLER *et al*. All these investigators have definitely differentiated this disease (of which the cause is supposed to be a virus) from spirochaetal jaundice as described by WEIL. At the same time careful search for Weil's spirochaetosis had invariably given a negative result. But history carries with it the description of outbreaks of such a disease in Egypt during the Napoleonic wars and in Gallipoli during the first World War (STITT, MANSON-BAHR). It is also stated that the disease is endemic in the Sudan and Egypt and on the shore of the Mediterranean. CAMERON in his excellent study of infective hepatitis in Palestine makes the surprising statement "cases of hepatitis, besides from Weil's disease which is endemic in that area" (quoted from the *Medical Annual*, 1944). Thus it appears to have been taken for granted that Weil's disease is endemic in the Middle East, but there appears to be no record of cases confirmed by the isolation or the demonstration of the spirochaete. Nor were we able to find records of cases confirmed serologically.

Not only is the lack of reports confirming the existence of Weil's disease in these areas striking, but it appears that when the disease was actually looked for, it was not found. Thus ALSTON and BROWN (1937) were unable to find it in Egypt, Arabia and Persia, while KIRK (1938) stated that its prevalence in the Sudan is doubtful as he failed to demonstrate it following the examination of 259 rats. In Palestine the disease was looked for by various observers. Extensive laboratory investigation, including animal experimentation (rats, guinea-pigs, and rabbits) failed to reveal the spirochaete. Our interest in the disease dates back to the year 1938 when a definite increase in the number of cases of infective hepatitis was observed. In order to rule out Weil's disease, several examinations, including guinea-pig and rat inoculation, were performed, all of them giving negative results. We therefore concluded that Weil's disease is probably non-existent in this country.

Our interest was renewed in 1944, when it became evident to us that two

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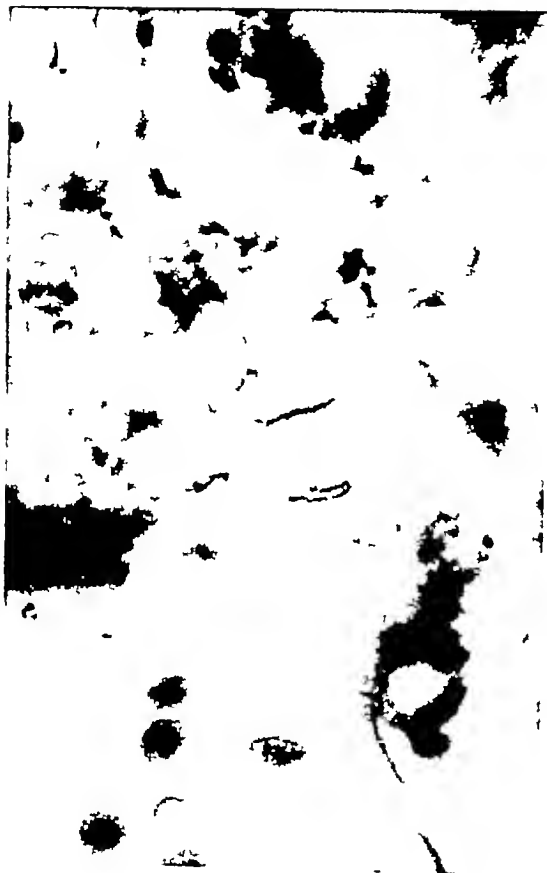


distinct clinical varieties of infective hepatitis existed, differentiated by the severity of the condition, the evidence of renal involvement, the leucocyte count and finally by the prognosis. It was also observed that the most severe variety appeared in persons connected with slaughterhouses—butchers, cattle farmers, etc. These observations led to a revival of the idea that leptospiral jaundice was existent in Palestine. On clinical grounds these cases could not be differentiated from Weil's disease.

During this time and while clinicians all over Palestine were investigating the cause of this disease, veterinary surgeons were concerned with epidemics of jaundice in cattle (ERZKIND *et al.* 1941; FARUND 1944). In connection with these epidemics BENCKOFF had succeeded in isolating a leptospira which was found to be serologically differentiated from *L. icterohaemorrhagiae* and *L. canicola*. Leptospiral jaundice in cows had been reported from Russia by MICHIN and AZIKOV (1935) and from Australia by JOHNSON (1943). Following this discovery it was decided to test the sera of patients recovering from the severe variety of jaundice. These sera were therefore sent to Dr BENCKOFF who found that though they contained no agglutinins against *L. icterohaemorrhagiae* and *L. canicola*, they contained on the other hand a high titre of agglutinins against the *L. bottis* isolated from cows in Palestine. As this leptospira was found to be non-pathogenic to rats and guinea-pigs, our failure to transmit the disease to these laboratory animals was thus explained.

Shortly after these findings, CASPAR and REIZ (1946) succeeded in demonstrating leptospirae in the liver and kidney of one of our fatal human cases, a butcher who had developed and died from a disease clinically identical with Weil's disease. (See Plate). In spite of repeated searches we were unable to isolate living leptospirae from the blood or urine of human cases. These findings clarified the poison and made it evident that we were dealing with a variety of Weil's disease caused by *L. bottis*. Consequently a considerable amount of research was stimulated all over the country. Sera from all suspected cases were tested. Patients who had been known to have suffered from this disease were traced and their bloods examined. It is unfortunate, however, that the agglutination titre falls rapidly and blood from cases who had recovered from the disease more than 1 year prior to the examination, gave negative results. Of special value was the work of CASPAR and REIZ who re-examined pathological specimens from the museum of the Beilinson Hospital with improved technique and were able to demonstrate the presence of *Leptospira* in organs of persons who had died several years previously. The results of these researches were reported, early in 1946 (*Haref* 30 No. 5), and are summarized in the following table which includes three additional cases observed by us and not previously reported.

At the time of writing, BENCKOFF succeeded in isolating leptospira from the blood of human case treated by Dr NATHANSON Haifa. Serologically this leptospira is identical with the leptospira isolated from cows. (Personal communications.)



*Leptospira bovis* in the liver of a fatal case of leptospirosis. Levaditi stain  
(By courtesy of Dr J Caspar)



TABLE I

Case number	Age	Sex	Occupation	Apparatus	Time of admission	Post-mortem examination	Result	Reported by
1	60	M	Butcher	—	—	Leptospirosis +	Died	BRIT & CASPAR
2	78	M	—	1700	1 month	—	Cured	BRIT
3	61	M	—	1100	7	—	—	—
4	76	M	Farmer	1200	14 days	Leptospirosis +	Died	CASPAR & RUI
5	45	M	Vegetable	—	—	—	—	—
6	75	M	Farmer	—	—	—	—	—
7	60	M	Butcher	—	1 week	—	Cured	PADERSKY
8	41	M	Shepherd	1000	2 months	—	—	—
9	47	F	—	1100	10 days	Leptospirosis +	Died	CASPAR & RUI
10	75	M	Farmer	Neurotic	—	—	Cured	PADERSKY
11	72	M	—	1000	—	—	—	—
12	—	F	—	1000	—	—	—	—
13	72	M	—	1200	—	—	—	IPATY
14	57	M	Butcher	1200	7th day	—	—	—
15	78	M	Farmer	1400	4 months	Leptospirosis +	Died	—
16	47	M	—	—	10th day	—	Cured	—
17	73	M	Butcher	1000	10th day	—	—	—
				1140	7th day	—	—	—

## MORBIDITY

The disease does not appear to be accompanied by a high morbidity. Thus during the year 1945 out of 1800 admissions to the Government Hospital, Tel Aviv which caters for the majority of the farming population of the district, only four cases were diagnosed serologically and one case by post-mortem examination. A similar state of affairs appears to exist at the Affiliated Hospital of the Workers Sick Fund, which caters for a similar population. Thus PADERSKY reported six cases treated at the above-mentioned hospital during the same period of time. Including the case reported by FIMRATI from Tiberias and one case from Tel Aviv (CASPAR and RUI) there were only fifteen proved cases in Palestine in 1945. Of course these figures are incomplete and further statistics have to be awaited.

## DISTRIBUTION

The disease was reported from the Sharon (Coastal) Plains by BRIT (1946) and from the Plain of Esdraelon and Jordan Valley, by PADERSKY (1946) and FIMRATI (1946). No reports of its existence in the Judea Mountains, Upper Galilee or Southern Palestine are as yet available. Actually the disease appears to be confined to the farming areas of Palestine and its distribution closely follows the distribution of cow pox.

## AETIOLOGY

Whether the leptospira found in man is identical with the cow leptospira is not yet decided, but serological tests appear to show that it is so. On the other hand this leptospira is serologically differentiated from *L. canicola*, and *L. icterohaemorrhagiae*. Thus though one patient's serum agglutinated BERNKOPF's leptospira in very high dilution (1:25,000), the agglutination of *L. icterohaemorrhagiae* was nil, and *L. canicola* was agglutinated in a dilution of 1:600 only.

## EPIDEMIOLOGY

The exact mode of transmission of the leptospira is still unknown. UNGAR (1946) calls attention to the fact that a calf artificially infected and killed 2 months after the infection showed a considerable damage of the kidney substance with the presence of enormous numbers of leptospires in the kidney tubules. Since this calf was clinically healthy UNGAR rightly concludes that spirochaetes may be eliminated in the urine long after the symptoms have disappeared and may therefore be the source of spread of the disease. The existence of healthy carriers may also be assumed from the fact that though some of the human cases were shown to have been in direct contact with jaundiced cows, in most of the cases no such contact could be proved. The condition is further complicated by the fact that the only sign of the disease may be a nephritis which in animals might well go undiagnosed.

The incubation period appears to be short. BERNKOPF found an incubation period of 10 days in the experimental inoculation of calves. PADERARY reports a case of leptospirosis in a man who fell ill 10 days after handling the organs of a cow dead of jaundice. Further researches will be required before a more definite statement can be given.

## SEASONAL AND SEX INCIDENCE

The disease appears early in the spring and the last cases are seen in the autumn. No definite cases have yet been reported during the winter months.

All the cases reported so far with two exceptions, occurred in males. Considering that this is an occupational disease, this incidence is not unexpected.

## CLINICAL PICTURE

The disease has a sudden onset characterized by chills, high fever, head aches, abdominal pains, sometimes muscular pains, and usually a marked injection of the conjunctiva. This period lasts from 3 to 7 days, during which time the patients though acutely ill, do not show any special diagnostic phenomena. At the end of this primary period, the fever drops to subfebrile levels and sometimes to normal levels. The patient becomes more toxic, icterus may appear, the tongue becomes coated and dry and the patient becomes stuporous and may even lose consciousness. This toxic stage may be very

ort (1 or 2 days) or may go on for several days. It is accompanied by a secondary rise of the temperature, and it may end in death. The following short outline of the clinical course of the disease as observed by us makes it clear that the disease is practically identical with Weil's disease.

### DETAILED SYMPTOMATOLOGY (See Table III)

- 1 *The fever* in the first period of the disease has no characteristic features, and if prolonged may be of the typhoidal type. In the toxic stage, the temperature usually rises rapidly just before death, as seen in the hepato-renal syndrome. In the cases that recover this secondary fever may last for 3 to over 30 days.
- 2 *The conjunctival injection*, which has been described as a diagnostic feature of Weil's disease, is here also very prominent. Whether it may be considered diagnostic or not, cannot as yet be decided.
- 3 *The icterus*, which first appears in the conjunctiva and then spreads to the whole body, is not usually very marked. The colour is pinkish yellow or orange rather than greenish. Therefore though the skin may not appear very icteric, the icterus index may be quite high.
- 4 *Muscular pains* are characteristic. They usually appear in the legs, back, or nape of the neck. Whether the severe abdominal pains may be considered muscular in character or as derived from a congested liver or gastritis is difficult to decide.
- 5 *Vomiting* is constant. It may be one of the first symptoms or it may set in during the toxic stage. In very bad cases "coffee grounds" vomiting occurs.
- 6 *Rash*. In two cases a scarlatiniform exanthema appeared in the toxic stage covering the whole body. But it was very short-lived, only lasting about 12 to 24 hours. In two cases a severe purpuric rash appeared 1 or 2 days before death.
- 7 *Oliguria* is almost constant in the toxic stage, in bad cases it may proceed to anuria.
- 8 *Blood counts*. This toxic stage is characterized by a definite leucocytosis which may be as much as 30,000 cells per cmm. The primary stage may show a leucopenia, or a normal leucocyte count. A marked thrombopenia was noted in two cases. (See Table II.)
- 9 *Urine*. A slight albuminuria may or may not be present in the primary period. The toxic stage in the more severe cases may show albuminuria, cylindruria, and haematuria. This evidence of renal involvement is of the utmost importance in the differentiation of this disease from infective hepatitis. It must be emphasized, however, that this is not a constant finding and may be absent even in fatal cases. Bile pigments usually make their appearance towards the end of the primary stage, and throughout the toxic stage. In one case the urine was loaded with tyrosine crystals.
- 10 *Blood*. The icterus index is elevated usually to a higher degree than one would expect from the clinical examination. The van den Bergh reaction becomes positive early in the toxic stage, at first the indirect type, but later the direct type. The blood cholesterol is unaffected. The blood urea is usually raised to very high limits in the toxic stage of the disease. This finding has led to statements being made as to the value of urea estimation in differentiating this disease from infective hepatitis. Though this is true in most cases, we have observed at least two definite cases where the urea was not elevated. The total plasma protein falls to the value of 5 to 5.5 mg per cent.
- 11 *Blood sedimentation*. This is usually normal or slightly elevated in the primary stage but very high in the toxic stage. The high level of sedimentation remains for several weeks after the recovery of the patient. In our opinion this finding is very characteristic as we had not met it in infective hepatitis.
- 12 *Haemorrhages*. The haemorrhagic tendency in the toxic stage is very characteristic. We have noted epistaxis, haematuria, melena, and subcutaneous ecchymosis (purpura). In two cases in whom purpuric manifestations were very marked, the platelet counts were only 7,000 and 32,000 per cmm respectively. The "coffee-grounds" vomitus referred to above, was found to be due to haemorrhagic gastritis. Further evidence of the haemorrhagic tendency has been found on post-mortem examination.

13. *Acute symptoms.* Signs of meningitis are often found but we were unable to demonstrate any pathological findings in the cerebrospinal fluid.

14. *Serological findings.* A slight positive agglutination for paratyphoid B is a common finding. This observation, quite puzzling at first, was later explained by an interesting coincident finding. In three cases, salmonella (*Salmonella enteritidis*), was isolated from the stools. This salmonella was found to be agglutinated to titre of 1:100 to 1:320 by the sera of the patients in our series. It is a well-known fact that salmonella infection in cattle in Palestine is very common. (Personal communication, Dr Bruckover). It appears, therefore, that the finding of paratyphoid B agglutination in these cases is due to the antigenic relation of *S. enteritidis* to *B. paratyphosus* B.

Specified *Leptospira agglutina* appear in the blood towards the end of the second week of the disease. The titre varies greatly and it may be anywhere between 1:1,000 to 1:25,000 or more. This titre falls rapidly and it becomes negative at the end of 1 year. In all the fatal cases the agglutination reaction was negative as it was taken before the 12th day of the disease.

TABLE II

DIFFERENTIAL WHITE BLOOD COUNT IN SEVEN CASES OF LEPTOSPIRINOSIS DURING THE TOXIC STAGE.

	Total.	Young and band forms.	Segmented.	Lymphocytes.	Monocytes.	Eosinophils.	Thick.	Banaphils.
1.	40,000	18	64	8	4		4	-
2.	12,000	23	34	4	8			1
3.	23,000	5	80	7	2		-	
4.	30,000	2	67	7	1		3	-
5.	18,000	8	77	10		-	8	
6.	23,000	15	64	8	7	1	5	
7.	18,000	3	84	8	3		8	-

TABLE III

SUMMARY OF CLINICAL AND LABORATORY DATA IN SEVEN CASES OF LEPTOSPIRINOSIS

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Fever							
Primary stage	4 d.	4 d.	7 d.	4 d.	7 d.	4 d.	8 d.
Toxic stage	d.	13 d.	4 d.	3 d.	25 d.	7 d.	3 d.
Conjunctival injection	+	+	+	+	+	+	+
Jaundice	+	+	+	+	+	+	+
Muscular pains	-	+	+	-	+	+	+
Vomiting	+	+	+	-	+	+	+
Rash	-	+	-	+	-	-	+
Leucocyte count	30,000	12,000	4,000	30,000	16,000	20,000	25,000
Blood sedimentation	92 mm	37 mm	83 mm	75 mm	45 mm	110 mm	80 mm
Blood urea	80	7	8	21	31	225	297
Duration of disease	6 d.	18 d.	14 d.	39 d.	45 d.	14 d.	13 d.
Result	Died	Cured	Cured	Cured	Cured	Cured	Died

### PATHOLOGY

The most important pathological findings as described by CASARI and RUIZ are the following —

- 1 *Liver* Cellular disintegration with perlobar round cell infiltration
- 2 *Spleen* Congestion
- 3 *Kidneys* Evidence of interstitial nephritis and tubular haemorrhages
- 4 *Other organs* Haemorrhages of mucous and serous layers
- 5 Leviditi stain may show the leptospira in the liver kidneys and other organs

### DIAGNOSIS

The diagnosis in the primary stage is no easy and rests on the history, the clinical findings and occupation of the patient. Attempts to demonstrate the leptospira by dark field examination and special staining methods of the blood have so far failed. The toxic stage can be recognized by the high leucocyte count, the rapid blood sedimentation rate, the evidence of renal involvement and especially the high level of blood urea, the characteristic conjunctival injection etc. At the end of the 2nd week of the disease the diagnosis can be definitely made by the agglutination of the specific leptospira by the serum of the patient. The presence of agglutinins can be shown on the 12th day of disease. The titre is low (1:300) and rises rapidly to 1:25,000. From then onwards the titre falls and it becomes negative after a period of 9 to 12 months.

The disease must be differentiated from infective hepatitis, cholecystitis, meningitis, septicaemia etc.

### COURSE AND PROGNOSIS

The course of the disease is short and stormy. In fatal cases death occurs between the 9th and 12th day of disease. The severity of the illness is not always a good indication of the prognosis as extremely toxic cases have dramatically recovered without special treatment while cases of less severity have died.

The cause of death is usually a hepato-renal failure (hepato renal syndrome) but one man who appeared to be on the way to recovery died in a very short time of a massive haemorrhagic pneumonia. Of the fifteen cases observed since the beginning of 1945 four died.

Convalescence is rapid. In most cases the whole course of the disease is from 17 to 20 days though in one case fever continued for a period of 45 days. The jaundice usually fades during the 3rd week of the disease and is not related to the fever. The same applies to the azotaemia. The blood urea usually falls to normal within 3 to 4 weeks.



## TREATMENT

Though we treated two cases with penicillin no definite statement can be given as to the response to treatment, as in both cases treatment was started during the toxic period. One of these recovered while the other one died. EPHRAÏM attributes the recovery in the case reported by him to penicillin. Further experience is required in order fully to appraise the action of penicillin on the leptospira. We have no experience with the serum treatment of this condition, as no serum is as yet available.

## CONCLUSIONS.

- 1 A condition clinically similar to Weil's disease exists in Palestine
- 2 The organs of fatal cases contain spirochaetal bodies while the blood of cases recovering from the disease contains antibodies against *Leptospira borda* but not against *L. icterohaemorrhagiae* and *L. canicola*.
- 3 The symptomatology and laboratory data of seven cases studied in this hospital and ten cases reported by other investigators is reviewed.

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# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

JANUARY, 1948

VOLUME 41

No 1

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**TRANSACTIONS**  
OF THE  
**ROYAL SOCIETY OF TROPICAL MEDICINE**  
**AND HYGIENE**

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**VOL 41 No 4 JANUARY, 1948**

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**LABORATORY MEETING**

of the Society held at the

**London School of Hygiene and Tropical Medicine, Keppel Street, London,**  
on

**Thursday, 20th November, 1947, at 7 30 p m**

THE PRESIDENT,

**SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,**  
in the Chair

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**DEMONSTRATIONS**

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**LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE**

**DEPARTMENT OF PARASITOLOGY**

**Prof H E Shortt and Mr W Cooper.**

**Staining of microscopical sections containing protozoal parasites by modification of McNameara's method \***

This method has the advantage of staining cells and protozoal parasites in tissue sections with colour effects resembling those seen in dry smears stained in Giemsa stain. Details of the technique are as follows —

- 1 Fix in Zenker, formol-saline or other suitable fixative
- 2 Embed in paraffin wax and cut thin sections
- 3 Remove wax with xylol
- 4 Remove xylol with alcohol.
- 5 Bring down to tap water

\* McNAMARA, W. L (1933) Giemsa stain for tissue, rapid method *J Lab clin Med*, 18, 752

- 6.\* Treat section with 5 c.c. Lugol's iodine added to 30 c.c. distilled water
- 7 Transfer to 95 per cent. alcohol.
8. Bring down to tap water
- 9 Treat with 0.5 per cent. sodium hyposulphate for 10 minutes.
- 10 Wash in tap water for 5 minutes.
- 11 Stain for 1 hour or longer in Giemsa stain, 10 c.c. acetone, 10 c.c. methyl alcohol, 10 c.c. buffered distilled water 100 c.c. (pH 7.2 to 7.4).
12. Wash momentarily in tap water
13. Differentiate in colophonium resin, 15 grammes acetone, 100 c.c. for 15 seconds or longer checking under the low power of the microscope. Renew solution as "film" forms.
- 14 Wash in acetone, 70 c.c. xylol, 30 c.c., with several changes.
15. Apply xylol without draining aside, then apply several fresh changes of xylol until section becomes clear
16. Mount in green euparal.

Examples of the use of the method in the case of various parasites were exhibited.

Prof H. E. Shortt and Mr W. Cooper

Low power preparation of living *Balantidium* from pig's laeas in the eighteenth sub-culture on Dobell's H.S.R.E. medium

Prof H. E. Shortt

Examples showing *Toxoplasma* sp. in different mouse tissues

Dr P. C. C. Garnham

Erythrocytic schizogony of *Plasmodium lach* in the liver of monkeys  
Sections and smears showing various stages in schizogony

Development starts in a parenchymatous cell of the liver the parasite showing peripheral distribution of nuclei. Later the nuclei become scattered, the surface of the organism convoluted and the cytoplasm vacuolated. A central space is formed which fills with fluid, with the eventual formation of the mature merocyst a body nearly 2 mm. in diameter containing innumerable merozoites in its rim. The cyst ruptures and the merozoites escape into the surrounding tissues and sinusoids, where the majority enter red blood cells, to become male and female gametocytes.

Dr P. L. Le Roux.

Twenty-four microscope preparations and 618 camera lucida drawings illustrating the shape and size of the eggs of species of schistosomes of man and animals.

---

In the case of tissues fixed in non-mercurial fixatives, steps 6 to 10 may be omitted.

## LABORATORY MEETING

## I INTRA-UTERINE EGGS OF

1 *Schistosoma haematobium* from man, Egypt and \*Southern Rhodesia  
The female from Egypt was incomplete, and in the specimens from the liver of an African (Southern Rhodesian) the ovaries were equatorial and not in the posterior third of the body, as is generally stated in textbooks to be the case. It would seem that the generally accepted morphology of the female is based on specimens recovered from experimental animals

2 *S. intercalatum* from \*man, French Congo (Prof. SCHUFFNER's material) and from sheep and \*mice, experimentally infected by Dr A C FISHER with cercariae which were believed to be those of this species

3 *Schistosoma* sp (probably *S. mattheei*)  
(i) "Unpaired" \*female from the liver of a white mouse, exposed to cercariae from *Physopsis africana* from Southern Rhodesia by Mr W ALVES. The eggs in this 50-days-old female appeared infertile and were of *S. haematobium* type but with abnormally long terminal spines

(ii) A \*female recovered by Dr F G CAWSTON from a guineapig, exposed to "wild cercariae" from *P. africana* at Durban. The general build and size of the eggs suggested that this schistosome was more closely related to *S. mattheei* than to *S. bovis*. It seems possible that some workers may take these eggs to be those of *S. haematobium* FAUST (1921) identified some of CAWSTON's material from the guineapig as *S. haematobium*, and notes that LEIPER (according to CAWSTON) had identified that the three male schistosomes, recovered by BECKER (1916) from a guineapig which had been injected with cercariae from *P. africana*, at Nylstroom, were the males of *S. haematobium*. The microphotograph, illustrating the male, suggests that the parasite was evidently the male of one of the species infesting sheep and cattle in the Northern Transvaal. There is no proof that *S. haematobium* has ever been reared to full maturity in mice and guineapigs

4 *Schistosoma mattheei* —  
Specimens from the veins of the urinary bladder of man and from cattle, sheep, goats, a baboon and experimental animals

5 *Schistosoma bovis* Specimens from cattle, game and experimental animals

6 *Schistosoma* sp (Closely related to *Schistosoma spindale*)  
\*Females from marsh buck, also known as situtunga or waterkudu (*Tragelaphus spekei selousi* Roth), cattle, red lechwe (*Cobus lechwe* Gray) and horse. This species has hitherto been recorded from Northern Rhodesia as *Schistosoma spindale* (MONTGOMERY, 1906). Comparison of these specimens with material from cattle from India and Malaya has revealed that the African species is distinct from the Indian

7 *Schistosoma spindale* \*Females from cattle in India and Malaya  
The species *Schistosoma spindale* var *africana* (PORTER, 1926), reported to have been recovered from a rat which had been exposed to cercariae, raised in *Planorbis pfeifferi*, from long spindle-shaped eggs from the urine of man, Transvaal, is by some authorities treated as a synonym of *S. spindale*

8 *Schistosoma margrebowiei* \*Female from red lechwe, Kafue flats, Mazibuka area, Northern Rhodesia  
The shape of the uterine eggs and the morphology of the female do not allow differentiation of this species from the Far Eastern one. It is generally maintained by some

\*These were exhibited under microscopes

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workers that differentiation of schistosomes can be based on the morphological characters of the females. In this species it is the males which permit differentiation. This is a parasite of cattle horses zebra, and various species of antelope inhabiting swampy pastures. It has been reported from man in the Belgian Congo, and there is reason to believe that it may invade man.

## II. EGGS FROM VARIOUS ORGANS OF THE BODY

In certain specimens from human tissue the size and shape of some of the eggs suggest infection with both *S. haematobium* and another species of *Schistosoma*.

## III. EGGS IN FECES AND URINE.

In certain of these specimens also, double infection of man with *S. haematobium* and another species with terminal-spined eggs was suggested.

Mr W. Alves.

Observations on *S. matthei* and *S. haematobium*. Adults and eggs from experimental animals and man

Textbook illustrations and descriptions of *S. haematobium* and its eggs are for the most part inaccurate. Many have, in fact, been copied *in toto* from the older works, e.g., Loom's, where *S. haematobium* is not differentiated from *S. mansoni*. The 7th edition of *Stitt's Diagnosis and Treatment of Tropical Diseases*<sup>8</sup> contains an interesting question of another kind. Page 1414 shows what purports to be an egg of *S. haematobium* but which is much nearer to the morphology of *S. matthei*.

BLACKIE's work in Southern Rhodesia on *S. matthei* infections in man was severely criticized by McHATTIE, MILLS and CHADWICK (wrongly in the writer's opinion) and, as a result, it has been largely neglected, although the relationship of the schistosomes bearing terminal-spined ova has continued to excite discussion, e.g. VAN DEN BERGHE.

In the writer's experience, while it is impossible to infect small laboratory animals with cercariae from *S. haematobium*, it is easy to infect them with those of *S. matthei* (infection in this context is intended to convey the eventual appearance of mature male and female schistosomes and their eggs.) It appears to be extremely probable that such work as has been described as on *S. haematobium* in these small animals has been based on a confusion between *S. haematobium* and *S. matthei*. [I must add here that I have not used hedgehogs (BRUMPT)] If this is the case, then the only large volume of experimental work of any value is that of FAIRLEY and MASON BAKER on monkeys.

On the other hand, *S. matthei*, and probably *S. botus* do appear capable of infecting man in Southern Rhodesia.

In view of VOGEL's work on the cross-breeding of *S. mansoni* and *S. japonicum*, an interesting hypothesis presents itself

<sup>8</sup>*Stitt's Diagnosis and Treatment of Tropical Diseases* (1944). 7th ed. Edited by R. P. Strong. Philadelphia: The Blackiston Co. London: H. K. Lewis

## LABORATORY MEETING

In human urinary schistosomiasis many eggs are seen which appear intermediate between "typical" eggs of *S. haematobium* and *S. matthei* (Le Roux's demonstration at this meeting may be quoted). Are they the produce of cross-breeding between *S. haematobium* males and *S. matthei* females?

Apart from anatomical possibilities, the female can, presumably, only oviposit where she is carried by the male.

It is hoped to follow up the question on the writer's return to Southern Rhodesia, where numerous monkeys can be obtained.

## DEPARTMENT OF ENTOMOLOGY

Prof P A Buxton

Slides made by Sir PATRICK MANSON (one of them dated July, 1894) showing development of guinea-worm larvae in *Cyclops*, also one slide of *Wuchereria (Filaria) bancrofti* in a mosquito. (All these slides were recently found in the Department of Entomology, London School of Hygiene and Tropical Medicine)

Three slides contain *Cyclops*. They are marked "Sir Patrick Manson's slides *Cyclops* infected with guinea worm," in the handwriting of the late Colonel A ALCOCK. One of them is also labelled, in handwriting identified (by Sir PHILIP MANSON-BAHR) as MANSON'S "Embryo entered *Cyclops* 27th July, 1894. Dissected 2nd September." One cannot now see any trace of nematode in this specimen, but the other two slides (not labelled by MANSON) contain *Cyclops* and nematode larvae.

One slide is marked in MANSON'S handwriting (*vide* P H M-B) "F nocturna, mosquito, advanced form".

The four microscope slides have been presented by the London School of Hygiene and Tropical Medicine to the Wellcome Historical Medical Museum.

The following comment is contributed by Sir PHILIP MANSON-BAHR —

MANSON'S work on the development of guinea-worm in *Cyclops*, which the three specimens exhibited amply demonstrate, was carried out in his "muck room" at 21, Queen Anne Street. The *Cyclops* used in his experiments he procured himself from ponds on Wandsworth Common. Others were sent him by friends in Birmingham. The guinea-worm embryos he obtained by his technique of "milking" from an Indian patient in the Albert Dock Hospital. The embryos were collected in watch glasses brought from the docks to his house and there introduced into bottles with a number of *Cyclops*. On observing them, some 24 hours later, he found that nearly every *cyclops* contained as many as ten to twenty guinea-worm larvae. According to MANSON'S observations, it appeared that the parasites had not entered the body cavity through the alimentary canal, but had penetrated the armour of the crustacean between the joints of the ventral plate. He found one instance, at least, was observed to live in *cyclops* for 70 days. The drawings and photographs made from these specimens at this period were preserved in Manson's Diary at Manson House and are now in the Museum of the School.

Prof LEIPER has stated to me verbally on several occasions that the stages figured in A P FEDCHENKO (1869) as those of *Dracunculus medinensis* are stages of *Cucullanus*.

workers that differentiation of schistosomes can be based on the morphological characters of the females. In this species it is the males which permit differentiation. This is parasite of cattle, horses, zebra, and various species of antelope inhabiting swampy pastures. It has been reported from mammals in Belgian Congo and there is reason to believe that it may invade man.

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## LABORATORY MEETING

This is perhaps the only fly that is specific to man, the adult feeding on his faeces and dejecta while the larva sucks his blood. The larva is remarkable for its power to resist starvation and desiccation. Larvae placed in dry sand and starved from the time of hatching lived for 28 to 33 days when kept at 25° C in a relative humidity of 70 per cent.

Notes appended to the exhibit dealt with this species' method of feeding, its growth and rate of development, its longevity and fertility, and its peculiar pairing and ovipositing habits. Short-focus photographs of the living larvae and adults by Mr W T BUSH were also shown.

## Dr Rajindar Pal

## The wetting of insect cuticle by spray droplets

The study of the wetting of insect cuticle by liquids has received little attention in spite of the fact that the effectiveness of all contact insecticides depends on the ability of spray liquids to wet the integument of insect pests. It is the object of this study to investigate the behaviour of spray droplets when applied to the integument of insects and make quantitative determinations of their wetting ability.

The wetting of a solid by a liquid is quantitatively expressed in terms of angle of contact of drops applied to the surface. If no wetting occurs, the drop remains approximately spherical and the angle of contact approaches 180°. Maximum wetting corresponds to zero contact angle and any intermediate ease of wetting is expressible in terms of some angle between these extremes. The apparatus and technique for measuring these angles was demonstrated. Some of the results are summarized below —

1 Wetting of insect integument not only varies greatly from species to species but also on different parts of the body of a single insect.

2 Legs and antennae are more easy to wet than the wings and the other parts of the body. This is very interesting because these observations independently support the common assumption that legs and perhaps pulvilli are the likely spots for the entry of insecticides when applied as a spray or as a residual film.

3 The physical nature of the integument, such as the general roughness and presence or absence of hair and scales, to a very large extent determines magnitude of these variations in contact angles. An interesting example afforded by mosquitoes and flies which are completely unwettable by water because of the covering of hairs and scales. It has been shown that the hydrophobic property of aquatic birds is largely due to the physical texture of the feathers and not due to the presence of oil or waxes as is commonly believed. The same is the case in animal furs and on the cabbage leaf.

4 Hard cuticular lipoids of *Tenebrio* are more hydrophobic than are the waxy type of waxes of blattids.

not of *D. malinensis*, and that therefore to MASON belongs the priority of having demonstrated the life-cycle of this human parasite in *Cyclops*.

The fourth slide, the section of mosquito thorax (*Culex pipiens*) from Amoy, China, demonstrating the development of *Wuchereria bancrofti*, was made by MASON in 1895. The history of this specimen which is figured in the first edition of *Mason's Tropical Diseases* (1896), Fig. 40, page 453, I believe to be as follows. On 20th June 1879, with a letter to COSGROVE (*Mason's Diary*), MASON forwarded a number of *Wuchereria*-infected mosquitoes preserved in glycerine. Whilst he was preparing the Gouldstonian Lecture for the Royal College of Physicians on the "Life History of the Malaria Germ Outside the Human Body" (1896), MASON was most anxious to obtain a *Wuchereria*-infected mosquito for microscopic section to demonstrate the method of development. He had written Ross in India and to friends in British Guiana to obtain specimens, without success.

In a letter to Ross dated 23rd December 1896, he related the following remarkable story: "I have at last succeeded in getting *Wuchereria*-infected mosquito. Many years ago I sent to COSGROVE a lot of *Wuchereria*-infected mosquito. I knew that COSGROVE's collection had gone to the Royal College of Surgeons, and got permission to look over them for my mosquitoes, but failing, I went to STEPHEN MACKENZIE; and there found a small bottle with a single mosquito floating in glycerine. In a section of the blood in its abdomen there were no *Wuchereria* filariae, and in its thoracic muscles there they were too—most beautiful to behold."

### Dr J. R. Buxvine

Behaviour of bed bugs under almost natural conditions. (The apparatus could not be brought to the Laboratory. Parties were taken to see it, leaving the Laboratory at 8.0 and 8.15 p.m.)

Two chambers, each 8 feet by 4 feet by 4 feet, made of materials used in prefabricated house construction, serve as miniature rooms. The crevices between sections are all sealed and special artificial bug harbourages provided which can be inspected without disturbing the insects. Every night, a pair of rabbits are put into wooden cages artificially infested with bed bugs.

Two types of experiment are in progress. (1) The migrations of the bugs from the original infestation site are being recorded. (2) The effectiveness of DDT wall treatments in exterminating the colony on the untreated rabbit cage is being determined.

### Mr C. Garrett-Jones (Introduced by Prof. P. A. Buxton).

#### Life history of *Aucklerymyia latrola*.

A laboratory culture was shown comprising all stages in the life-cycle of *Aucklerymyia latrola* F., the African muscid fly whose larva is known as the Congo floor maggot.

The specimen is figured (Fig. 17) in these Lectures (1896). *Brit. med. J.*, 1896, 2, 712, 744.

† The Life and Work of Sir Patrick Manson (1927), p. 32.

† The box containing six phials of mosquitoes was ultimately found intact in the Royal College of Surgeons in 1935 by Prof. R. T. LEAVER, and is now in the Museum of the London School of Hygiene and Tropical Medicine. So MASON missed them, and they were there all the time.

† Sir STEPHEN MACKENZIE, Physician to the London Hospital, who lived in Cavendish Square, H. had corresponded with MASON and had done the first work on reversal of filarial periodicity in 1891.

## LABORATORY MEETING

of paludrine in urine. In response to requests, this has now been further simplified to permit its use under the most primitive of field conditions.

It has been found that the concentration of the various reagents is immaterial to the final colour. The only accurate measurements needed are of the urine, the standard solution and the extracting solvent. The volume of the latter need not be known provided that it is the same for all estimations—it should be about 10 ml. A graduated test-tube will suffice for the measurements. A pipette is required for the urine and standard solutions.

In the absence of a balance, the solutions may be prepared as follows —

**Standard**—A 100 mg tablet is dissolved in a small volume of dilute hydrochloric acid and made up to 1 litre with water.

**Copper reagent**—1 volume saturated copper sulphate solution, 2 volumes saturated ammonium chloride, 40 volumes water.

**Caustic soda**—5 volumes saturated sodium hydroxide solution, 95 volumes water.

**Sodium diethylthiocarbamate**—Saturated solution.

The procedure is exactly as described by GAGE and ROSE, and the colours obtained are compared visually with those in a series of standards similarly prepared containing in place of urine

	0	0.3	0.6	1.0 and 1.5 ml standard solution
1 e, 0	30	60	100 and 150 $\mu$ g drug	

## DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY

Prof R M Gordon and Mr W Crewe

The bite of the tsetse

The generally accepted idea of the behaviour of the mouth parts of *Glossina* during the act of feeding appears to be that of a rigid tube piercing the skin and dermal layers and ending in a blood vessel, from which the blood is then withdrawn. In this method of feeding the majority of the metacyclic trypanosomes emitted from the hypopharynx of the feeding fly would be carried away in the general circulation.

A study of the specimens shown in the present demonstration suggests that the action more closely resembles that previously described in the case of *Aedes aegypti* (GORDON and LUMSDEN 1939) *Ann trop Med Parasit*, 33, pages 259-278. The fascicle is capable of being curved during the act of piercing the skin and, in the cases so far observed, feeding is in the nature of "pool feeding," 1 e, the taking up of blood from a previously formed haemorrhage, which in the case of the guinea pig lies just above, and sometimes in the muscle layer.

Slide 1 was a section of the leg of a guinea pig, taken 1 hour after a tsetse bite, showing the "pool" of blood caused by laceration of the blood vessels while Slide 2 was a similar section, taken 24 hours after the bite, showing t

*Blood films.*

- 1 Normal rabbit red cells.
- 2 Fowl blood with *P. gallinaceum*.

3 A method of using Leishman stain for these sections.

*Method.*

These paraffin sections are brought down to distilled water and are then covered with Van Gieson for 5 to 10 minutes. This stains cellular tissue yellow and connective tissue pink. The stain is then washed off with water and the slide covered with Leishman stain freshly diluted with an equal quantity of distilled water. This is allowed to act for 20 to 25 minutes. The section is then differentiated with running water for 10 to 15 minutes. Excess water is then removed and the section dehydrated through a series of xylol-acetone mixtures,

5 per cent. xylol 95 per cent. acetone.  
30 per cent. xylol, 70 per cent. acetone.  
70 per cent. xylol, 30 per cent. acetone.  
xylol.

The section is mounted with a neutral mounting medium.

*Sections*

- 1 Kidney fowl, exo-erythrocytic forms of *P. gallinaceum*.
- 2 Liver monkey central necrosis *P. knowlesi* infection.

Dr G T Stewart and Mr W B Jones.

*Studies in the pathology of experimental amoebiasis*

Microscopic sections, photo-micrographs and tabulated data were shown, illustrating the influence of intestinal bacteria upon an experimental amoebic infection in the rat. Briefly the results showed that bacteria were intimately concerned with the early development and character of the amoebic lesions. Thus certain strains of *Bact. coli* and *paracolon* were found to increase the severity of the lesions and evidence was given to suggest that one or other of these organisms played a major role in every case. When large doses of penicillin were given, sufficient to inhibit these resistant bacteria, the severity of the infection was significantly reduced. Penicillin acted both as a prophylactic and therapeutic agent and its action was enhanced by phthalyl sulphathiazole. This latter drug, given alone, had some slight prophylactic action but no therapeutic effect. Detailed results of these experiments are at present in the press.

Miss M M Tottley and Prof B G Macgregor.

*Field method of estimating pabulins in urine*

GAGG and ROSE (1946) developed a simple procedure for the estimation

GAGG, J C. & ROSE, F L. (1946). *Ann trop Med Parasit* 40 333

## LABORATORY MEETING

subsided Since 1945 he has also had intermittent haematuria, and on numerous occasions has also brought up variable amounts of blood *via* the mouth

At the Hospital for Tropical Diseases, moderate numbers of active *Entamoeba histolytica* (together with scanty *Trichomonas*) mixed with erythrocytes, pus and epithelial cells, were found in various specimens of his urine and seminal fluid The blood which he brought up at irregular intervals *via* the mouth also contained numerous active *E. histolytica* Repeated examinations ( $40\times$ ) of his faeces failed to reveal any amoebae or cysts of *E. histolytica*, and sigmoidoscopy ( $2\times$ ) failed to show any abnormality

Smears of urinary deposit, seminal fluid, and "oral" blood were fixed with Schaudinn's fluid and stained with haematoxylin Examples of these preparations, revealing typical *E. histolytica*, many of which contain ingested erythrocytes, were shown in the demonstration

With a view to finding the sites of the amoebic lesions, a thorough investigation of the patient was carried out While the precise origin of the "oral" blood eluded determination, the investigations, including urethroscopy, indicated the "urinary" lesion to be in the bulbar part of the urethra

### Dr W E Kershaw, Dr P J O'Meara and Dr G T Stewart

#### Observations on amoebiasis in Ceylon

Stools were examined from 1,229 Asiatic food-handlers in Ceylon, using wet film and copper sulphate flotation methods Eleven per cent carried *E. histolytica* cysts, the range being from 9 per cent in some districts to 13 per cent in others Of these cases, 40 to 56 per cent were diagnosed from one specimen and 80 to 92 per cent from four specimens Among healthy Europeans the carrier rate was 5.8 per cent Amoebiasis was the most important single cause of hospital admissions for diarrhoea, though in certain seasons its incidence was exceeded by that of bacillary dysentery

In two naval messes, the removal of infected native food-handlers was followed by a decline in fresh cases and readmissions of amoebiasis

### Mr J A Lock (introduced by Brigadier J S K Boyd)

Stained slides and a living preparation of *Toxoplasma* sp in tissue culture from material supplied by Professor Shortt

Tissue cultures of embryonic rat heart infected with *Toxoplasma* sp were demonstrated The tissue was grown 4 days before infection with a drop of peritoneal exudate from an infected mouse

Slides and photomicrographs showed rapid multiplications of the parasites within the tissues After 12 hours, several divisions had taken place, and 24, many cells contained "rosettes" of sixteen or more parasites The centrioles were readily seen after Schaudinn fixation and Giemsa staining



persistence of the uncoagulated haemorrhage. Slide 3 was a section of skin showing the marked curvature of the proboscis of the tsetse during the act of feeding. The specimen was obtained by snipping off the proboscis of a feeding *Glossina*.

Dr F Hawking and Dr W L M Perry (National Institute for Medical Research)

*Plasmodium knowlesi* maintained in frozen condition for 5 months

Various American workers have described the preservation of malaria parasites in a frozen condition at  $-76^{\circ}\text{C}$ . for long periods, e.g. 12 months. This technique is being used at the National Institute for Medical Research for the preservation of a strain of *P. knowlesi*. Blood containing very many schizonts of *P. knowlesi* was removed from a monkey on 17th May 1947 and heparinized. It was placed in  $\frac{1}{2}$  c.c. ampoules and frozen by immersion in a mixture of solid carbon dioxide and alcohol. It was then stored at  $-76^{\circ}\text{C}$ . (by the kind co-operation of Dr F FULTON). Successful preservation of the parasites depends upon rapidity of freezing and of thawing: the biggest mortality among the parasites occurs during this transition phase. On 6th November 5½ months later the ampoule was thawed by placing it in water at  $37^{\circ}\text{C}$ . A smear from the blood showed that most of the parasites had retained an approximately normal appearance. The rest of the blood was injected into a monkey which showed parasites in the peripheral blood 6 days later.

In a previous experiment, blood infected a monkey after having been stored for 5 months.

This technique for storing parasites is very convenient because —

1. It saves the labour and expense of animal or human passages, which would be particularly useful for strains of human malaria.

2. It prevents any mixing with other strains which are being maintained in the same laboratory.

COOKEHILL, L. T. (1939). *Proc Soc exp Biol Med* 43, 499.

MAXWELL, R. D. (1945). *Amer J Trop Med*, 23, 123.

WOLFROCK, F. (1945). *Amer J Hyg* 42, 155.

Dr C A Hoare and Dr F Murgatroyd

An unusual case of amoebiasis.

The patient was admitted to the Hospital for Tropical Diseases, London, in September 1947. He had travelled extensively in the Middle and Far East during the last 9 years. In 1945 he suffered severely from diarrhoea, which was diagnosed as amoebic dysentery and responded to anti-amoebic treatment, but relapsed a year later. Following further treatment, the diarrhoea gradually

## LABORATORY MEETING

He was given 43 grammes of urea-stibamine with excellent immediate results

On 24.4.46, on his arrival in the United Kingdom he was afebrile, but his spleen was readily palpable. He was given a full course of sodium stibogluconate in spite of a negative sternal marrow smear and culture.

On his return from sick leave, his spleen was still palpable and leishmania were found in his sternal marrow smear and grew out on culture. A further course of sodium stibogluconate was given, and from this time he was subjected to further courses of pentostam and pentamidine with only temporary benefit (Chart was shown).

In July, 1947, he showed a spleen enlarged some three fingers' breadth below the costal margin. A sternal marrow puncture revealed no leishmania, but a splenic puncture 4 days later was full of leishmania. This slide was shown. It was considered that the spleen was the reservoir of the infection and it was decided to start a course of carbo-stibamide and, in the middle of the course, to perform a splenectomy. This was carried out by Air Commodore P. A. HALL, and recovery was uneventful apart from a superficial haematoma. This course of carbo-stibamide was completed after the splenectomy and followed in 12 days by a full course of pentamidine. Sternal marrow smears at the end of the course, and on 16.11.47 were negative and so far are sterile on culture. Patient has remained completely afebrile since 9.9.47. His red cell count, which was 3,000,000 in July prior to splenectomy, is now 5,000,000, but he has a lymphocytosis of 64.5 per cent in a total cell count of 8,400 per cmm, and this is the only disquieting feature. There is no lymphadenopathy or enlargement of the liver, and he states he is feeling very well. He will be kept under careful observation for a further 6 months.

The interesting points are —

1 The fallacy of negative sternal marrow smears, especially in cases under treatment

2 The extreme value of a spleen smear where marrow smears and cultures are negative

3 In a case steadily retrogressing in spite of treatment, it is worth while to carry out a splenectomy, if splenic smears are positive and marrow smears consistently negative, while the patient's general condition is still satisfactory

4 The spleen weighed 5½ lb and was full of leishmania

Dr F Murgatroyd

Two patients suffering from leprosy under treatment with sulphetrone were demonstrated

A living preparation was shown under a phase contrast microscope and the intracellular parasites were readily seen.

(The phase contrast microscope was kindly lent by the makers, Messrs. Cook, Troughton and Simms.)

#### Wing-Commander Ian MacKay

*Fluorescence microscopy for demonstrating the tubercle bacillus. A technique for counting T.B. in dispersed fluid culture*

The apparatus demonstrated is a modification of that described by LEWERT (1944). The ultra-violet light source is a 125-watt mercury vapour lamp made by B.T.H. (mercuria) or G.E.C. (omra) operated from A.C. mains and wired in series with the appropriate choke. An electric fan (ventaxia) is included in the circuit the whole being enclosed in an asbestos-lined wooden box, baffled at the ends. The microscope sits on top of the light housing and the mirror discarded, focusing of the light being done by manipulation of the lamp.

Placed in the body of the microscope is a yellow gelatine filter (Ilford No. 109 "delta"). The optical system consists of  $\frac{3}{4}$  inch and  $\frac{1}{4}$ -inch objectives and a  $\times 5$  eyepiece. Preparations are stained with suramine.

Preparations shown

1. Positive T.B. sputum.
2. B.C.G. 5 days growth in Dubos liquid medium.
3. Human T.B. 5 days growth in Dubos liquid medium.

#### *A technique for counting T.B. in dispersed fluid culture*

A measured drop (1/100 ml.) of the fluid to be examined is placed on an albuminized slide. It is stained by suramine and, using the fluorescence microscope, every organism present is counted. Two mechanical aids are used, a special counting eyepiece and an electrically operated counting machine. The latter makes possible the simultaneous counting of the total number of organisms and the total number of clumps a clump for this purpose being considered an aggregate of one or more organisms.

#### Air Commodore T. O. Morton

*A very resistant case of kala-azar apparently cured by splenectomy followed by pentamidine*

A corporal, R.A.F., contracted kala-azar in Calcutta, and was diagnosed a month after the onset of symptoms by a positive sternal marrow smear on 29.1.46.

LEWERT H. (1944). Fluorescence microscopy in the detection of tubercle bacilli. *Lewert*, 23rd December page 818.

## LABORATORY MEETING

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Two patients suffering from leprosy under treatment with sulphetrone were demonstrated

Dr M Yoell Dr A T Roden and Dr J D Abbott.

- 1 Smears from subcutaneous nodules of a male showing microfilariae  
(?) *Onchocerca* sp
- 2 Developmental forms of (?) same species in *Anopheles sachwevi* and  
*A. maculipennis typicus*

The specimens were obtained in Eastern Macedonia, near the mouth of the Struma river during July and August, 1948

Developmental forms of filariae were observed in 3 per cent. of 450 dissections of *Anopheles sachwevi*, and in one specimen of *A. maculipennis typicus*. The numbers of filariae varied from one to eight per mosquito.

No clinical or pathological evidence of filariasis was found in the local human population.

Local horses and mules were observed with subcutaneous, or deep cutaneous, nodules, similar to those previously reported from Greece by PAPADANTZ (1936). Specimens of microfilariae from these lesions were exhibited

## ORDINARY MEETING

of the Society held at  
Manson House, 26, Portland Place, London, W.1  
on

Thursday, 11th December, 1947, at 7 30 p m

THE PRESIDENT,

Sir PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P  
in the Chair

**The President** Tonight we are to have a paper by Dr J L McLETCHE, and when you have once seen him you will never forget him. He is here tonight to tell us the results of 12 years hard labour in Nigeria. I am informed that he is the greatest authority on this subject which he has made his own, but he has been so modest as to cloak himself in a cloud of obscurity. He has given credit to everyone but himself for the work he has done.

### PAPER

## THE CONTROL OF SLEEPING SICKNESS IN NIGERIA

BY

J L McLETCHE, M B , C H B , D T M & H \*

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\* I have to thank the DIRECTOR OF MEDICAL SERVICES, Nigeria, for permission to publish past and present members of the Sleeping Sickness Service for advice and assistance. Dr FISHER of the Geography Department, Aberdeen University, for making maps, and the staff of the Tropical Diseases Bureau for obtaining references. The photographs reproduced by courtesy of the Central Office of Information.

Almost 20 years ago, BAKU'YIN (1928), wrote in an Italian journal "In Europe far too little is realized of the vast amount of work performed by colonial medical officers in waging war against parasitic diseases and in particular against trypanosomiasis. Your continued interest in the problems of sleeping sickness since that date, and for years before, shows how little such a statement ever applied in Britain.

Earlier this year in his Chadwick Lecture, Professor VAN HOOFF gave credit to his two co-workers equally with himself. I can go much farther. From 1929 until his appointment as Director of Medical Services, Palestine, Dr LESTER was responsible for sleeping sickness work in Nigeria. To his foresight and to his energy in producing results with a relatively tiny staff is to be added the accurate research and field work of Dr NASH, our entomologist for many years. What credit remains is divisible between the many medical officers, entomologists, control officers and sleeping sickness officers who served in the field, often under very trying conditions between administrative and technical officers who advised and assisted them and between the African staff who served us all. I am merely the recorder of their work.

#### INTRODUCTION.

The Nigerian Sleeping Sickness Service, a semi-autonomous branch of the Medical Department, is directly responsible for all field work in controlling human trypanosomiasis, and indirectly responsible for the treatment of hospital cases, in that it provides the drugs required and advises on treatment. The outlook and policy of the service has always been coloured by its origin, in 1921 as the Tsetse Investigation under Sir WALTER JOHNSON. At no time since then has there been neglect of the ultimate aim of gaining permanent control, in all possible areas, by curbing or eliminating the insect vector.

Nigeria is an enormous colony with a very large but relatively poor population. Twelve years ago, the allowance for all medical and health work was about fourpence per head per annum. Roughly tenth of this was devoted to anti-sleeping sickness measures. This was just sufficient for senior officer five or six medical officers, each of whom had his African team for mass treatment, one entomologist, and one technician and for the maintenance of limited research, mostly entomological. For 2 brief years, 1937 to 1939, thanks to the Colonial Development Fund, the number of European officers, never all on duty simultaneously and with majority inexperienced, rose to possible twenty-five. Thereafter the staff position steadily deteriorated, and was at its worst in 1946. During the war years it became rare to have in the field more than eight officers, some of them seconded and inexperienced. At the same time, in addition to its specific functions, the service undertook steadily increasing amount of medical and health work in rural areas, organized medical and dispensary service for specially recruited mines labour and was periodically mobilised to assist in controlling outbreaks of meningitis, smallpox, yellow fever and relapsing fever. In periods of emergency one felt that much of the sleeping sickness work was something that had to run automatically while all available energy was concentrated elsewhere.

After years of fluctuation, the Service has been constituted, since April, 1947 on a more permanent basis, with an establishment of sixteen officers. Experienced European and African staff released from the Forces, resumed full duty

J. L. MCLEITCH

s year, and vacancies are being filled. In outlying areas with low infection rates, the new mobile field units of the Medical Department will assist in investigatory surveys and in supervising treatment. Native administrations are beginning to take over some of our medical and health work.

Local research, at a very low ebb since the closure of Gadau, and now much needed, will mostly be undertaken by the new West African Trypanosomiasis Research Institute, originally sponsored by LESTER (1945).

Subject to the vagaries of colonial finances, and to the absence of interruptions lasting as long as the recent war, future reports on Nigerian work should be of accelerating progress, particularly as regards vector control.

## I DISTRIBUTION OF TSETSE FLY-MAN CONTACT

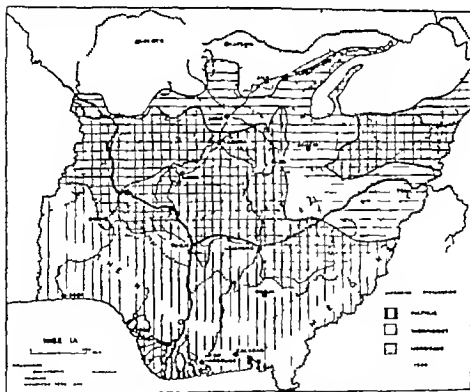
About 10,000,000 of Nigeria's people live in the densely populated Southern Provinces, where a variety of tsetse species occur, but where the known sleeping sickness areas are small and confined to the Eastern Provinces and Cameroons. Of the 12,000,000 people in the more spacious Northern Provinces, one-half live in areas where sleeping sickness has been reported. The map, p. 448, modelled on one prepared by Dr. NASH in 1943, shows the approximate zonal distribution of the three important vectors of the trypanosomiasis, *Glossina morsitans*, *G. palpalis*, and *G. tachinoides*. The two latter, the riverine species, are the important carriers of the human infection. *G. tachinoides* occurs alone to the north where it can survive the hostile climate in remarkably scanty shade. Requiring greater rainfall and heavier cover, *G. palpalis* is found to the south. Centrally, their distribution overlaps. Recently NASH has compiled for the Colonial Office a very full report on tsetse in the four West African colonies, which I hope will soon be made available.

More important than the superficial distribution or the actual density of fly is the degree of its contact with man, or rather, of its repeated contact with man. ROUBAUD (1915) was among the first to point to the high incidence of sleeping sickness where tsetse were not abundant. In Nigeria, TAYLOR (1931) and NASH (1944) described extremely close contact with a few highly infected tsetse in shaded villages and at a water-point. Departmental reports frequently mention fly actually in or at villages, rest-houses, markets, fords, mine workings, washing places, water-points, and other frequented places. Much infection has been attributed to the spread of fly during the rains into shaded villages (LESTER, 1938). HARDING (1940) believed that "continuous and close proximity with the fly seems to have been necessary for anything more than casual infection."

In two widely separated areas (in the Katagum Division of Bauchi and the Anchau area of Zaria, where control work was to begin) it was found that there was approximately 1 mile of stream to each square mile of country. This figure probably applies to much of the Northern Provinces. It is also a common finding that practically the whole length of a stream has enough vegetation



to harbour fly at some time of the year. For instance, both POLLARD (1912) and JOHNSON and LLOYD (1923) instanced the presence of *G. tachinoides* where the only shade was scanty mimosa. Even in heavily settled and farmed country in the north, this species can persist along streams in and close to towns, and may even live in the overgrown moats of old walled towns, as it did at Rano and Anchau. In the last 40 years, a steady movement of dispersal took much population from the relative safety of the old towns to new hamlets and homesteads which were too often sited near the most permanent pools of infested streams. The African's habits are no negligible factor in determining close and repeated contact. He seeks shade and water as do the tsetse, and often at a time of day when the latter are most active. Conditions conducive to a persisting high degree of contact between man and the riverine tsetse are therefore common, especially in the Northern Provinces.



MAP L.—Approximate Zonal Distribution of *Glossina morsitans*, *G. palpalis* and *G. tachinoides*.

*G. morsitans* although an efficient carrier of *T. gambiense* in the laboratory is of no importance in the field. In Northern Nigeria it is associated with patches of low population and high game density. There is no evidence either of its spread or of its connection with outbreaks of sleeping sickness.

## II THE TRYPANOSOME DRUG RESISTANCE

Local research regarding the virulence of Nigerian strains of *T. gambiense*, their pathogenicity, response to drugs, and transmissibility by tsetse, occupied only a few years and virtually ceased with the closing of Gadau. The important results communicated by LESTER in *Annual Reports* between 1930 and 1934, and in a paper (1933) have since been largely confirmed in the Ivory Coast, French Sudan, Fernando Po, and, most fully, in the Belgian Congo by VAN HOOFF and his colleagues (1938). VAN HOOFF (1947) adopts LESTER's classification of strains of *T. gambiense* into three categories. These are —

1 Trypanosomes of low virulence but high transmissibility, occurring in new foci or epidemic spread. They produce in man a mild infection characterized by slow development, slight symptoms and scanty blood infection. Tryparsamide resistance is rare.

2 Trypanosomes of medium virulence, invading the nervous system in 6 to 12 months. Arsenic resistance varies widely but the majority of cases respond to tolerated doses. Methodical arsenical treatment may favour selection of resistant strains which persist.

3 Trypanosomes of high virulence, resembling *T. rhodesiense*, very resistant to tryparsamide but feebly if at all transmissible, especially in relapses after treatment.

Both LESTER and VAN HOOFF agree that arsenic resistance is common and that it exists as a natural characteristic of *T. gambiense*, that the role of the individual host in producing or increasing this resistance is important, that routine or mass treatment either does not, or does not inevitably, increase it, although irregular treatment may do so, and that the chronic advanced patient, especially after some treatment, is little danger to the community.

The bearing of these findings on treatment is highly important. By 1934, that is, as soon as possible after these conclusions had been reached in Nigeria, treatment with tryparsamide alone ceased, and combined treatment, antrypol (suramin, germanin, moranyl) first in full doses, followed by tryparsamide, was given as a routine to all patients, early, advanced and relapsed.

In pre-antrypol days, resistance to tryparsamide was common in a few areas (LESTER, 1933). HARDING (1945) records that, 18 months after treatment with up to 30 grammes of tryparsamide, nearly half of a group of Plateau patients were either dead or still infected. In most such areas there had been irregular previous treatment. It is difficult to estimate the incidence of arsenic resistance now that patients are usually rapidly sterilized with antrypol and laboratory facilities are non-existent. In 1946, of seven Zaria cases given twelve 2-gramme doses of tryparsamide, but no antrypol, two were still infected 5 months afterwards. A group of twenty-five Katsina cases on tryparsamide alone (24 grammes) were all negative at the end of treatment.

Resistance to antrypol appears to be rare. It has been stated that it does not occur in man, but VAN HOOFF (1938) mentioned a few cases. Two of his own cases were resistant to antrypol and to tryparsamide, but both strains were non-transmissible. Dr HOLLINS, formerly of the Sleeping Sickness Service, in a very wide experience saw two cases trypanosome-positive at the end of standard treatment with antrypol and tryparsamide. It is extremely rare by ordinary methods of finding trypanosomes in the blood or glands of cases relapsing

after our standard treatment. YOKER and his colleagues (YOKER, MURGATROYD and HAWKING 1933 LOURIE and YOKER, 1938), working with *T. brucei* and *T. rhodesiense* did not record a double resistance to any two of the three drugs now in common use in Nigeria—antypol, the diamidines and trypanamide.

Where resistance to arsenicals is frequent, VAN HOOFF *et al.* (1938) advised the use of antypol or a similar drug as a public health measure. VAN HOOFF later (1947) recommended that it should be given to all early cases and to sterilize arsenical resistant cases whose trypanosomes are still transmissible. This is tantamount to advising antypol or a similar drug in all cases, since neither arsenic resistance nor transmissibility can be determined in the field.

### III. INCIDENCE AND DISTRIBUTION OF SLEEPING SICKNESS.

Before 1925 sleeping sickness in Nigeria, as in other West African colonies, was considered to be quiescent and sporadic.

Typical annual returns from the Northern Provinces were of seven, eight and fifteen cases in 1909 1910 and 1922 respectively. SCOTT MACLEOD (1911) examined over 8,000 people in parts of the present Zaria, Niger and Benue Provinces. He found that about 7 per cent. had some enlargement of cervical glands few of which were puncturable and none positive—one blood film in about 300 had trypanosomes. In the same areas very high infection rates were found between 1930 and 1940 and today are around 1 per cent.

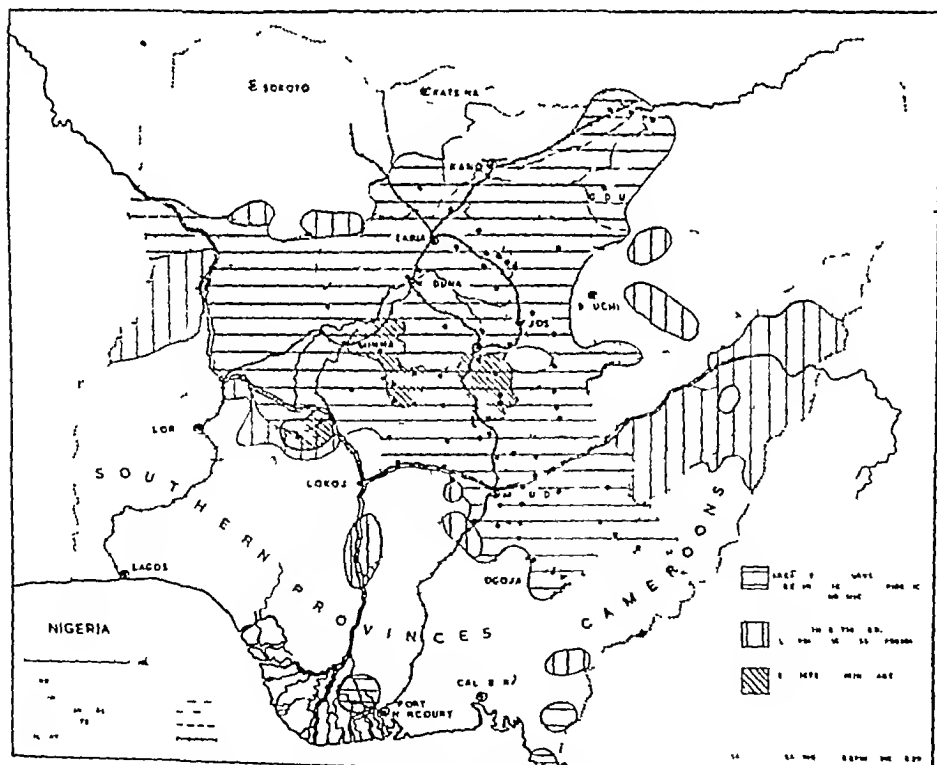
In 1925, Sir WALTER JOYNSON at the first Kaduna Tsetse Fly Conference, reported an increase of the disease in the Northern Provinces, adding that this was due not to spread of tsetse but to dispersal of population, freedom of movement, and the employment of large labour forces in mining and railway construction. These three factors have been subsequently stressed by other Nigerian writers (LESTER, 1933, 1945 HARDING, 1940). From 1928 to 1930 itinerant medical officers treated about 3 000 cases annually and, by the latter year hospital cases had risen to almost 2,000. The epidemics were still thought to be localized to parts of three provinces, but further work revealed similar conditions elsewhere (LESTER, 1933). As in other colonies, increased activity and knowledge resulted in increased awareness and higher returns but there had undoubtedly been what LESTER (1933) described as "a disconcerting spread."

In 1930 the first survey team was organized. Itinerant voluntary treatment was replaced by regularized mass treatment. A few years later five or six teams were at work and by 1940 the bulk of the primary surveys required had been completed.

The daily routine of these teams has been described by LESTER (1945) and HARDING (1940). It was similar to that of the French teams except that as a rule only the simpler techniques of gland puncture and blood examination (fresh blood or thick film) were used for diagnosis—treatment was standardized and not based on individual C.S.F. or other findings, such as response to single large dose of trypanamide and all team members engaged in both diagnosis and treatment, there being no separation into diagnostic and treatment teams. More complicated techniques would have delayed progress and required an enormously greater staff.

The primary surveys done by the teams fall into three periods. In the first 5 years infection rates averaging 13.6 per cent (1,246,039 people, 169,440 cases) were found in the more central northern provinces. The position was serious. For instance, Zaria Emirate had a 20 per cent rate and this was causing steady depopulation. In the next 5 years, the extent of the infected areas was more clearly delimited. Although the average 1936-1940 incidence was 8.6 per cent (1,510,304 people, 130,560 cases), the higher rates were still central, including much of Benue Province, but moderate figures (1 to 5 per cent) were common at the edge of the main belt. In the outlying provinces, Adamawa, Ilorin, Kabba and parts of Bauchi, investigatory surveys showed small foci or sporadic cases, although tsetse conditions were much the same as in the central epidemic areas. In the last 6 years (1941-46) new surveys have been few and mostly peripheral, their average finding 1.5 per cent (445,952 people, 6,946 cases).

Map II outlines the areas covered by mass surveys and reconnaissance surveys. The distribution of dispensaries treating sleeping sickness shows



MAP II —Distribution of Sleeping Sickness and of Dispensaries treating Sleeping Sickness

their concentration in the badly affected central provinces, and heavy concentration in the two more northerly of the specially restricted mining areas. Thus there is only one dispensary any significant distance west of the Lagos-Ibadan railway—it treats only two or three cases a month. The six dispensaries near the western border of Banchi Province also treat relatively few cases. The units with high annual returns are almost all in Zaria, Plateau, and parts of Benue Provinces.

In the Southern Provinces, there have been, since the original focus at Eket described by SCOTT MACFIE (1913), severe but localized outbreaks in Owerri and near the Cameroonian coast. In parts of Ogoja and the interior of Cameroons moderate infection rates are known and are now being dealt with.

#### IV CHARACTERISTICS OF THE DISEASE

##### DIAGNOSIS PROGNOSIS: TREATMENT PROPHYLAXIS.

Trypanosomiasis of man, as seen in Nigeria, has been described by LESTER (1933, 1938, 1945) and classified into the same three broad categories as the common trypanosome strains, mild, nervous and toxæmic. Even in virulent epidemics the mild form predominates. LESTER (1939a) also noted that the proportion of severe nervous cases in Uganda, Congo and Sudan was not much higher than in Nigeria, but SAUNDERS (personal communication) informed me that such cases seemed to be fewer in Nigeria than in Gold Coast.

The use of the term "mild" is relative. It conveniently covers both a great number and a great variety of cases. The other two possibilities, rapidly progressive nervous involvement—the classical *gambian* sleeping sickness—and acute toxæmia, are both extremely serious conditions, and have been fully described in the literature.

At the first glance, the striking thing about many mild cases is their apparent normality and good health. In most this is apparent only. In 1911 KINCHEORN described Gold Coast cases of low virulence—13 months later nearly half of them were dead. Despite the predominance of mild cases, LESTER (1939) mentioned a 12 per cent. fall in adult male population in Zaria Emirate in the 10 years preceding mass treatment. In the same province, HARDING (1940) confirmed the correlation between death-rates and sleeping sickness incidence, not in an area where cases were obviously ill, but in a district where more than half the cases appeared in normal health, only six in 3,000 were advanced, and not one was dying at the time of diagnosis. In a neighbouring district, at a village where 50 out of 500 people had died within a few months, I found 15 per cent. of the remainder to have trypanosomiasis of apparently the mildest type, almost all having normal or only slightly modified spinal fluids. In Uganda, the *gambian* infection is also described as mild, but fatal (BROWN 1938). One must remember that it is the trypanosome of low virulence that is specially associated with epidemics, and there can be no

question of the damage that such epidemics cause. Advanced cases and *rhodesiense*-like infections are often rare. It is the mild cases that give a high proportion of the deaths, and the clinical condition of the majority of patients may bear no relation to the devastation being caused.

Close or prolonged observation does, of course, reveal a fairly definite syndrome in these apparently healthy cases. Symptoms are usually periodic and patients are frequently incapacitated temporarily. With high infection rates the economic loss to a community may be very great. The swollen, stupid, or rather sad expression of early sleeping sickness cases is characteristic. Hypotonia, slight facial asymmetry, insomnia, rheumatic pains, impotence and some loss of strength are common.

A moderate incidence of trypanosomiasis may exist without evidence of serious effect on the public health, but no one can guarantee the stability of such a condition. In a Katsina district, despite an infection rate of 11 per cent, HARDING (1940) found no correlation between incidence and mortality. In both this and in a neighbouring district, infection rates of 0.9 per cent were found in 1946. Among 853 cases left untreated for a period averaging between 3 and 4 months, 90 died. HOPE-GILL (1930) described wide variations in severity in two different areas, contrasting the apparently fit and healthy Pankshin pagans with the more severely affected Hausa people of Birnin Kudu in Kano. Today sleeping sickness is rather more common and more deadly in the same area of Pankshin than it is in Birnin Kudu.

That there are truly mild cases is undoubted. TODD (1924) described long survival, from 9 to 13 years, of eight untreated cases. In Ilorin I have found trypanosomes in gland juice after prolonged search in people who gave a clear history of very slight but typical symptoms, lasting between 5 and 8 years, and tending to decrease or actually disappear. Such cases appear to have established an equilibrium with their parasite. In how many this state would be reached, or how many would go on to spontaneous recovery, we do not know. HOLLINS and LEWIS-FANING (1947) have described a 20-month follow-up of Nigerian patients. Thirteen still had trypanosomes and their condition was deteriorating, but ten had no trypanosomes discoverable by routine field methods and appeared well. Some of the thirteen positives had at times been apparently trypanosome-free and well, but they eventually broke down. This might also have happened to some of the ten negatives, although others might have remained well permanently. In the same area many of the control cases given a single dose of antypol appeared to be cured at the end of 20 months. I have seen similar effects following a single large dose of pentamidine.

Regarding the serious depopulation associated with high incidence of mild trypanosomiasis, HARDING (1940) followed LESTER (1938) in attributing many deaths to lowered general resistance and intercurrent infection. For some years, I have felt that a very considerable proportion of deaths, apart from advanced cases, arises from acute toxæmic exacerbation of the actual

trypanosomiasis. The mild infection may be slowly progressive as regards involvement of the nervous system, but sudden breakdown, with high fever and toxæmia, occurs in patients who have had the usual minor manifestations of the disease for months or even years. HOLLINS and LEWIS-FANTINO (1947) describe one such example in a carrier. Most of these cases die unseen and untreated. Some recover but their after history is shortened. From the numbers I have seen, and from the histories obtained from survivors or from relatives, this sudden loss of equilibrium must be relatively common. Its occurrence makes all the more urgent the search for and the early treatment of, the common mild case, in which treatment is not sought voluntarily. The deaths in the Katsina cases I quoted show that even 1 per cent. of a mild infection can be harmful if treatment is not available.

### DIAGNOSIS AND PROGNOSIS.

Under present conditions of low endemicity diagnosis of about 90 per cent. of survey cases is made by gland puncture, which should be repeated several times if necessary. Examination of blood films gives fewer positive results than under epidemic conditions. There is no evidence of the presence of numbers of blood positive carriers without glandular enlargement. In endemic areas increased cells or protein in the C.S.F. is regarded as diagnostic. Relapses present a special difficulty. After the exhibition of antrypol it is rare to find trypanosomes in gland juice or blood. In relapses and in a varying proportion of new untreated cases—a proportion which may be very high in some areas—diagnosis must be clinical or by lumbar puncture.

HOLLINS has shown the value, in diagnosis and prognosis, of a simple method of estimating the 10-minute erythrocyte sedimentation rate (HOLLINS and LEWIS-FANTINO, 1947). The normal African rate varies in different areas, but a pronounced fall in an abnormal rate after administration of antrypol is diagnostic. Dr MARSHALL CHALMERS, now of St. George's Hospital, confirmed that the same very rapid 10-minute fall occurred when Wintrobe's tubes were used.

In prognosis the state of the C.S.F. is most important. Increase of total protein is of more significance than cell increases in deciding treatment. If the C.S.F. is abnormal after treatment the cell count is the more delicate indicator of cure or failure of treatment (HARDING 1945). Using the Sicaud Canteloube method of estimating total protein (normal up to 22 mg per cent.), heavy dosage of trypanamide is required for amounts over 30 mg and the outlook is poor with initial readings much above 40 mg per cent.

### TREATMENT

All, or almost all, early cases can be cured by antrypol, pentamidine, or butarsen. None of these drugs can be used alone without an enormous increase in staff to lumbar puncture all patients and eliminate those with nervous involve-

ment Antrypol usually sterilizes the blood for very long periods and has a prolonged prophylactic effect (HARDING, 1945) The value of such a drug from the public health point of view is very great In the advanced stages of trypanosomiasis tryparsamide is still the only safe and effective drug, but its very moderate trypanocidal activity (BROWN and PEARCE, 1924, YORKE, 1940), plus the possible frequency of resistant strains, seem to make it quite unsuitable for use alone in the field It is interesting to note that, whereas workers in *T gambiense* areas recommend combined antrypol-tryparsamide treatment, rather than tryparsamide alone, because of arsenic resistance, MACLEAN and FAIRBAIRN (1932) advise the same combination, rather than antrypol alone, because *T rhodesiense* has strains or phases sensitive to arsenic As contrasted with the successive use of these two drugs, their simultaneous use is believed to involve a synergic boosting of the therapeutic value of the smaller doses usually given Synergic treatment was advised by EHRLICH many years ago and has been favoured by French workers, especially in chronic cases (SICÉ, 1937)

The standard regime given by teams doing mass treatment in Nigeria totalled antrypol 3.2 grammes, followed by tryparsamide 10 grammes, in nine injections given at intervals of 5 days The first injection was of 0.2 gramme antrypol as a test for idiosyncrasy In most areas 80 to 95 per cent of survey cases were cured In Sierra Leone the same course, or modifications of it, gave a cure rate of 93.5 per cent (HARDING, 1945) At dispensaries an additional five 2-gramme injections of tryparsamide were given The use of a synergic antrypol-tryparsamide mixture (0.5 gramme antrypol, 1.5 gramme tryparsamide) is now more common, six to eight injections being given in early infections, and up to 20 injections to advanced cases Its advantages are that survey cases obtain the same amounts of the constituent drugs in 30 instead of 40 days, dispensary cases who attend for only 40 to 50 days, as many do, receive rather more of each drug and get their tryparsamide early, irregular attenders get antrypol at each visit, in chronic cases the results are rather better In Nigeria our patients normally stand up to these various courses very well, unlike HARDING's (1945) sensitive Sierra Leone cases Our death-rates during treatment rarely exceed 1 per cent, blindness is less than 1 in 1,000, impaired vision after treatment about 1 per cent, and exfoliative dermatitis is very rare I have seen three cases only

Pentamidine could replace antrypol Provided that it is given by intramuscular injection only, it is safe It appears to have a slight action in nervous cases, though this is difficult to prove, and it is a prophylactic HARDING (1945) finds its combination with tryparsamide non-toxic, and Nigerian experience confirms this For large numbers of survey cases daily treatment may be no advantage (HARDING, 1945), but a short intensive course is useful for small groups of mild cases in remote areas For these we recommend pentamidine isethionate, 100 mg, the 1st day then 200 mg daily to the 7th day, together with tryparsamide injections totalling 6 to 9 grammes in the same 7 days, or



in 10 days. Where many patients are intolerant to antrypol, pentamidine is indicated. Otherwise there is little to choose between them and a final decision would rest mostly on their respective keeping qualities and cost.

Butarsen alone is effective only in early cases. There seems no point in combining this trivalent arsenical with trypanamide in advanced cases, and in our experience the combination is not so good as synergic antrypol and trypanamide.

Two arsenicals now on trial are melarsen and melarsen oxide, but another year will elapse before the results can be properly evaluated. Melarsen has, I believe, been discarded elsewhere as being too toxic, but so far we have observed no undue toxicity. It gives good immediate results and in two instances rapidly sterilized patients who appeared trypanamide-resistant. Melarsen oxide is powerfully trypanocidal but very toxic in advanced cases. It sometimes fails completely in early infections. In nervous involvement the C.S.F. cell count may be rapidly reduced, but after 6 months the relapse rate is one in three. This drug is not suitable for use in the field. It may find a place in the controlled treatment of selected advanced cases in hospital.

A drug combining all the properties of antrypol and trypanamide and improving on the action of the latter in severe nervous cases has yet to be found.

In the great majority treatment is straightforward and effective: most effective when diagnosis is early and when attendance for the all-important initial course of treatment is regular. The few seriously ill toxæmic or very advanced nervous cases require careful handling and react badly to high dosage. A great reduction in the number of chronic relapsing cases, so common at some dispensaries and hospitals, follows on early diagnosis, regular treatment, and early review to ascertain the need for a second course of treatment.

### DRUG PROPHYLAXIS

A trial of antrypol as a prophylactic in mining camps gave certain protection for 6 weeks but not for 3 months. It was not considered justifiable to expose healthy labourers to the slight but definite risk associated with antrypol for such a brief protection (LISTER, 1938, 1945). VAN HOOFF and his associates (1944) have shown that pentamidine gives longer protection, and VAN HOOFF (1947) advises its general use to reinforce the effects of mass treatment. In small-scale experiments in mining camps since 1944 we had not, up to the end of 1946, found a case of overt or cryptic infection in those given pentamidine, even up to a year previously although some labourers left the area after shorter periods and may of course, have developed infection after leaving. In the restricted mining areas of Plateau Province, two-thirds of the 3,500 labourers are now being protected regularly being given pentamidine isethionate, 250 mg., every 4 or 5 months.

### V GENERAL PRINCIPLES OF CONTROL

The general principles of control, by chemotherapeutic, administrative or anti-tsetse measures, have been recognized for over three decades.

In a review in the *Sleeping Sickness Bulletin* in 1910, BAGSHAWE stated that even if an effective drug was found, control would be uncertain until cheap and effective means were found of suppressing tsetse, exterminating it in one area, reducing it in another. Twenty years ago principles of control were clearly enunciated by various committees and commissions. French recommendations underlined the value of agricultural and administrative measures, and the importance of protection from tsetse, of resettlement, improved dietary and general welfare. Chemotherapy was then recognized as the soundest basis for the prevention of sleeping sickness. It still is, but it should be regarded as the foundation only. Where it seems easier to control the population than the ubiquitous tsetse fly, there is a danger of relying too exclusively on chemotherapy.

In Nigeria, when JOHNSON and LLOYD first reported the increase of sleeping sickness in the Northern Provinces and advised on staff for treatment and tsetse control, they affirmed that, in the areas concerned, fly could be controlled or eradicated by clearance and resettlement. Within a few years of the start of the mass treatment campaign, LESTER (*Annual Report, 1934*) reaffirmed the acceptance of these principles. Immediate control was to be by chemotherapy, ultimate and more final control, where possible, by extensive campaigns of protective clearance made and maintained by communal effort. As a last resort, where these measures failed, concentration of population might be necessary. In later papers elaborating this policy (1938, 1939a, 1945), LESTER emphasized above all the economic and public health aspects of control. The primary aim is the permanent prevention of deadly epidemics such as were experienced in the past. When, however, sleeping sickness is brought to a low endemic level, it may have no greater effect on the public health or economics of a country than have a variety of other endemic diseases. Then expenditure on further measures of chemotherapeutic control must be balanced against the benefits accruing.

In controlling epidemics, chemotherapy is our strong right arm. In new foci and in epidemic extensions, the common trypanosome strains are most amenable to treatment. In all but exceptional areas in Nigeria we now know that efficient mass treatment, repeated if required, or fortified by the provision of permanent treatment centres, will in a few years reduce sleeping sickness rates from a dangerous to a reasonable level. There is still a grave potential danger, even if the infection appears mild. To maintain or further to lower the incidence by treatment alone is expensive, requires a constant vigilance which cannot be relaxed, and may prove obnoxious to some tribes. Finally, such control is palliative and not permanent. LESTER (1938) always insisted that the only certain and permanent method of control is to reduce or eliminate man-fly contact wherever this is possible.

The primary chemotherapeutic aim is to control epidemics. The secondary, the inherent right of the individual sleeping sickness patient to receive treatment as fully curative as is possible, is not neglected. The ideal is to combine, with the minimum upset to both the patient and the community, plus the minimum burden to the Treasury, the preventive sterilization of the carrier and his full restoration to normal health. As yet we have no single drug which will do this, and no combination which will do it rapidly in all cases.

Concurrence in LESTER's views, comes from such authorities as DE BRAUWERE (1938), of the Belgian Congo Foreami, and from VAUCHEL (1943) in French Cameroons. The latter confirms that a low incidence of trypanosomiasis does not affect population figures and hardly interferes with economic activity. More recently VAN HOOFF (1947) has cited the failure of very intensive treatment to eliminate infection. He recommends regular prophylactic injection for the whole of the non-infected population. In common with past policy we would reserve this rather onerous restriction for special areas, such as mining camps, where sleeping sickness may be an industrial disease of grave danger.

#### RESULTS OF MASS TREATMENT

The organization and routine work of the Nigerian survey teams have been described by HARDING (1940) and LESTER (1945). The latter also pays high tribute to the role of dispensaries in consolidating the gains won by mass treatment. The chain of dispensaries had its beginning in 1934 when the possibility of doing regular or annual resurveys of endemic areas, as is done in other colonies, had to be discarded. In war time, when team work ceased for a time except in one province, the number of dispensaries was increased. There were, in 1946, eighty-nine dispensaries and some temporary dressing stations which provided treatment for sleeping sickness. Of the dispensaries, fifty-one were run wholly by the Sleeping Sickness Service, which also supplied staff to twenty native administration units. Lord HAILLY (1939) stresses that the mere multiplication of facilities for the treatment of such individuals as apply is not enough when dealing with mass diseases. The Nigerian sleeping sickness dispensaries have always been regarded as centres from which the dispensary staff senior African staff and touring officers, should do periodic resurveys in the area served. Low dispensary returns are not always sufficient evidence that infection rates are not rising and they must be backed up by the more definite information given by resurveys. This function, as regards dispensary staff gradually faded in most districts when medical, health and anti-epidemic work grew burdensome, but is now being successfully revived, especially as native administrations take over their share of the general work.

In 1938 it was planned to assign experienced medical officers to certain provinces to follow up declining epidemics and treated cases, but the war intervened. For long periods there might be one medical officer in the field, and there were seldom more than two. Once, for 3 months, there was only one for both field and headquarters work. The sole area regularly to have a medical officer was Benue Province, which HOLLIES and LEWIS-FANTING (1947) describe as about the size of Scotland, with 1,200,000 inhabitants. Senior African staff supervise small regions with up to six dispensaries, conduct resurveys and follow up cases.

The present tendency is for most resurveys to be done by small teams

visiting all principal villages. Attendances for examination are usually good, and may be over 95 per cent with European supervision, but normally no drive is made to enforce 100 per cent attendance except in special cases, *e.g.*, high incidence, or a known resistant focus. In districts where work by full teams met with passive or active resistance before the war, good results have been recently obtained by small sub-teams, whose work is inspected and checked, but not constantly supervised by European officers. This system is much easier for the peasant, the native administration officials, and our small European staff, and much larger numbers over wider areas can be examined.

The dispensaries have always been popular, particularly in pagan areas, and the proportion of trypanosome-positive early or moderately advanced cases is often high. In recent years, with decreased supervision, many patients attended irregularly or incompletely. Luckily the standard dispensary treatment catered for the more advanced infections, and cure rates remained reasonably high. There is, however, especially in the old endemic areas, usually a steady decrease in the number of fresh infections, with a relative increase in the proportion of chronic relapsing nervous cases requiring prolonged treatment. This may be due to selection of strains (NAPIER, 1946, VAN HOOF, 1947).

From the public health point of view, it is not economic to have staff immobilized in giving long courses to advanced but epidemiologically non-dangerous patients, while numbers of more or less healthy carriers are at large in the area. The principal function of dispensary staff is now to co-operate with native administration headmen in doing frequent village surveys. The early dangerous cases and the early relapses are then brought in regularly for treatment under the wing of the hamlet head, who is responsible for them. In Zaria Province the proportion of relapsed cases under treatment has come down from about 40 to 18 per cent. (This figure is not the relapse rate.) At one dispensary, where the local cases have been followed up, the proportion of relapsed to all local cases under treatment has fallen from 45 per cent to nil in 3 years. As checks on dispensary figures, touring officers see groups of cases before treatment and do spot surveys in the same districts.

The global figures (Table, p. 460) are a reasonable yardstick for measuring progress. Since 1931 half a million cases have been treated. The 1946 resurvey figures are again below 1 per cent for the first time since 1939, and infection rates found by teams or by dispensary staff are similar. The 1946 sampling was adequate, half a million people having been examined in areas scattered throughout eight provinces. In one Katsina district (Galadima), where past survey had been unsatisfactory (LESTER, 1939), and where there had been neither dispensary facilities nor protective clearing, the infection rate was recently 2.8 per cent (2,004 cases in 70,658 people). Similar, though smaller, foci occur in other provinces. Resurveys have mostly been made in areas of Katsina, Zaria and Benue where original infection rates were from about 10 to 20 per

Sleeping Sickness Service								General medical stations	Total cases treated in Nigeria
Year	New surveys		Infection rate per cent.	Re-surveys, by teams, at camps and at dispensaries.		Infection rate per cent.	Cases treated at dispensary.		
	Population examined	Cases		Population examined	Cases				
1931	100,200	8,104	5.0	—	—	—	—	3,488	8,572
1932	148,971	12,202	8.2	4,127	641	19.4	—	2,747	16,780
1933	223,712	23,122	11.2	18,214	2,797	14.4	—	1,228	22,257
1934	206,222	41,428	11.2	12,446	1,221	10.8	—	4,612	47,428
1935	407,202	21,264	20.8	—	—	—	209	5,024	86,587
1936	419,216	47,019	11.4	7,219	281	9.9	10,480	4,021	62,721
1937	427,707	26,708	6.4	5,651	252	2.8	2,187	4,460	41,646
1938	508,411	18,027	9.1	169,428	2,026	1.2	4,272	4,272	26,016
1939	442,746	18,100	6.8	220,821	1,914	9	9,625	9,625	30,091
1940	186,060	17,467	6.2	68,641	1,879	2.1	6,824	7,729	22,149
1941	203,123	2,684	1.8	60,272	1,042	2.1	9,825	2,414	17,202
1942	186,686	2,810	1.2	82,745	1,625	2.1	11,212	1,866	17,294
1943	19,918	424	2.1	218,778	9,427	2.2	12,100	2,702	21,664
1944	—	—	—	T 114,749 M 42,400 D 4,709	2,242 479 (466)	2.0 1.1 1.8	10,422	2,280	19,929
1945	228	8.4	1.2	T 100,848 M 42,782 D 72,516	1,174 322 (1,141)	1.2 0.7 1.6	19,126	2,197	25,224
1946	—	—	—	T 257,721 M 23,448 D 115,879	2,802 227 (1,080)	1.0 0.8 9.0	9,707	3,286	16,722
Total	2,272,281	29,916	9.9	1,229,224	27,742	1.7†	110,909	22,620	499,240

T = Teams, M = Missions, D = Dispensaries.

Does not include the dispensary cases shown above in brackets.

† The 2,642 dispensary re-survey cases for 1946-48 are added to the 27,742 team and mission cases in calculating the infection rate.

cent and in some cases much higher. There have been no reports of increasing incidence from the edges of the old endemic area. Staff is now available to ascertain the exact position in these provinces. A resurvey in Owerri in the south revealed very few cases.

Annual dispensary returns are fairly constant around 10,000. In each of the last 2 years more than a thousand of these have been early infections brought in after village surveys. We hope to increase the proportion of these. The sharp rise in 1942 and 1943 to 13,000 dispensary cases corresponded with the opening of numbers of new dispensaries. The average annual return from dispensaries used to be over 200. It is now fairly steady at about 110. The value of the dispensaries is shown by the fact that they treat 60 per cent or more of all cases in the colony.

Hospital figures show some increase but will probably fall as they did after 1948, following extensive surveys. Work this year in districts round two hospitals with consistently high returns gave low infection rate around and below 1 per cent. Special staff is posted to such hospitals to continue resurveys, to co-operate with native administrations in ensuring regularity of treatment, and to relieve hospital staff of a heavy burden.

These results may not seem spectacular when compared with the low infection rates obtained in other colonies. Our very small treatment staff has however, accomplished its set task of reducing a deadly epidemic to safe proportions without imposing undue restrictions on the people in general. It should further reduce incidence as the entomologists and vector control workers extend permanent security.

The view of LESTER (1945) was that in most areas an infection rate of about 1 per cent would have little effect on general mortality. He added the important provisions that population figures must be satisfactory, that there be no signs of increase in the disease, and that permanent treatment facilities be available. These are still Nigerian views. We add to them that there must be the positive evidence of resurveys that no increase is occurring, and that full and proper use must be made of the facilities we provide. The onus of arranging attendance for examination and for regular treatment is of course, on the native administrations.

#### CONTROL OF LESTER

Organized research began in 1921. Before this some interesting work had been done and, as early as 1909, there had been a trial of what we now call partial or discriminative clearing, *i.e.*, the removal of undergrowth and the thinning of larger trees.

The semi-arid climate of the northern parts of Nigeria is so unfriendly to tsetse, which barely survive the hot season by retreating to permanent pools and dense shade, that limited or partial clearance may upset the delicate balance (NASH, 1940). At present we are dealing principally with *G. tachinoides*, which

can exist in a very narrow fringe of riverine shade, as may be seen in the picture of a clearance gang at work (Fig 2). Towards its northern limit, clearance against *G. palpalis* is practicable and effective (LESTER, 1945).

NASH (1937) described two lines of approach, the defensive and the aggressive. Defensive, protective or "rod" clearings are as used in other colonies to reduce penetration of fly at fords, water points, etc. (BROWN, 1938; GIBBINS, 1941). Since high infection rates depend on very close contact with fly well planned clearings will give a high degree of protection to the population in the course of their normal occupation. Clearings must be well maintained to give permanent protection. NASH recommended clearance of 300 yards on either side of a danger spot as the minimum for *G. tachinoides* and 400 yards for *G. palpalis*. In practice, 400 yards is frequently used in pure *tachinoides* country. Clearings may be extended and linked up. They must be ruthless: no trees are left, but grass is not cut. The application of protective measures throughout a large district of several hundred square miles demands considerable local knowledge and great attention to detail. A single district may require over 200 individual clearings each at least 800 yards long.

Aggression entails the complete elimination of tsetse from an area. NASH accomplished this at Anchau by a combination of partial clearance throughout the river systems plus barrier clearings along all streams at the border of the tsetse-free area. The barrier clearings, which are essential to prevent re-invasion from untouched main rivers outside the area, are ruthless, a mile long and extend back 50 yards from the stream banks. To maintain extensive work of this nature at least seventy people to the square mile are needed. In many areas of Nigeria this would entail concentration of population.

In 1935 the outlook as regards control of sleeping sickness was gloomy. High infection rates were still being found both at primary surveys and at the few resurveys which had then been done. The dispensary system was in its infancy and outlying provinces had not yet been investigated. There was no trained staff for tsetse control, although the principle of effecting protective clearance by communal effort was accepted and some native administrations had done useful work under the guidance of medical and administrative officers. Local approval was obtained for a scheme of general expansion of sleeping sickness control and a grant of £95 000 was given from the Colonial Development Fund, conditional on the expenditure by Nigeria of an extra £55 000 over and above her normal annual expenditure of about £25,000. The grant was to be spread over 5 years, but this was extended to 10 years. The scheme of expansion included improvement of treatment facilities, provision of staff to plan and supervise a greatly increased campaign of protective clearance, and funds for a small programme of aggressive clearance involving some concentration of population. For this last, improved village layouts, sanitation, markets, water supplies and other measures of general development were regarded as essential from the beginning. The main long-term policy was mass treatment



FIG 1 —Typical S S cases (gland juice positive) Blood being taken for estimation of E S R (Ogoja Province)



FIG 2 —Clearance against *G tachnoides* Note narrowness of fringing forest

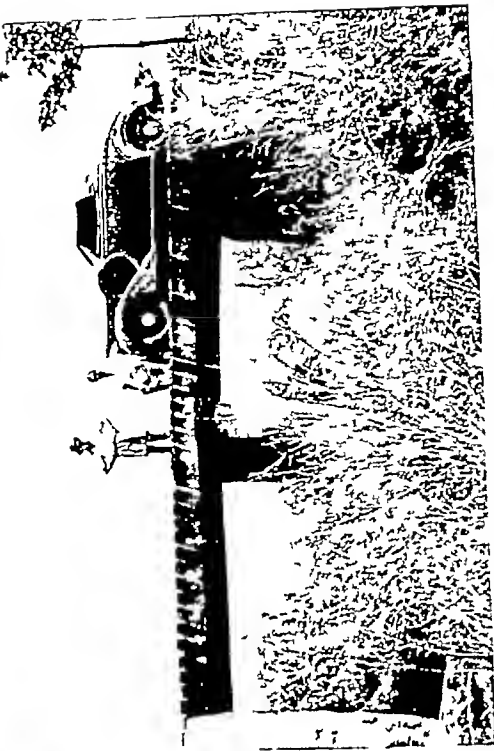


FIG 3 —Bridge built by S S Service over ruthlessly cleared stream



FIG 4 —Takalafiya (New Anchau)





5



7



6



8

FIG 5 — Standard wheelbarrow rollers and basins.

FIG 6 — Anchoa Propaganda Team. Village Koorvachider being interviewed.

FIG 7 — Anchoa Village School. Normal teacher on right.

FIG 8 — Village School. Abdul Chav.

plus protective clearance. The scheme of concentration and resettlement in tsetse-free conditions was an experiment that had to be undertaken lest other measures failed and large-scale concentration became necessary. In the event, by the time the scheme started in 1937, treatment was producing good results, the freedom from infection of many provinces was known, and it could be surmised that concentration would rarely be required.

The staff for tsetse control and resettlement was to number fourteen: two entomologists, an administrative officer, a water supply foreman, and ten control officers, most of whom had agricultural qualifications. Although the resettlement scheme was controlled by the Sleeping Sickness Service, there was an advisory committee of senior departmental officers, and local departmental officers gave much advice and assistance. The Emir of Zaria was frequently consulted, and successive Chief Commissioners took a close interest in the work at Anchau.

### PROTECTIVE CLEARINGS

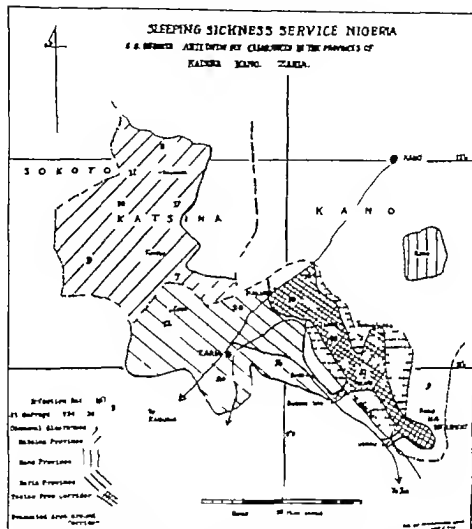
Work by the new control officers did not begin until 1938, and by 1941 what staff remained had to be concentrated, with great reluctance, on Anchau. Two years later protective clearance was resumed, and by 1945 so much had been done that the remaining staff, now three control officers only, was almost fully occupied in maintenance of old clearings.

Map III, p. 464, shows some of the areas of protective clearance in Katsina, Zaria and Kano Provinces, and their relation to the Anchau tsetse-free corridor. Very extensive clearings have been made in parts of Kano not shown, smaller areas in Bauchi and Plateau have been controlled, and old clearings in Sokoto re-examined. The Kano work has been largely a re-clearing of streams originally done by the native administration many years ago. Protection has been given to about half a million people inhabiting some 6,000 square miles. Not every hamlet in a district can be protected, some have too small a population to make and maintain the clearings required. The only solution is to move them, or to pay for the clearings if the local population can maintain them.

As an instance of the work done, thirteen villages in the Galadima District of Katsina were protected in early 1946. Infection rates in 1945 had been from 6 to 18 per cent. The total population was 6,700, of whom 1,800 were adult males or youths. Twenty-three clearings totalling 10.6 miles were made. This would take about 10,000 man-days with paid labour but the villagers reported that it took them 17,700 man-days. That is only 10 days per adult male or youth. Re-slashing required 5,000 man-days in 1946-47 and will become less yearly. The shade along the rivers was heavier than the average for Katsina. Fly was found in only one clearing in the 1946 wet season.

The regular inspection of the very numerous clearings made is a heavy burden, and must continue for up to 10 years from the original date of clearing. In provinces where extensive work has been done, it is now agreed that the native administrations will have some staff and funds for tsetse control.

The efficiency of these "rod" clearings in reducing fly at danger spots is undoubted. Their effect on infection rates is becoming clear in the districts where protection has lasted some time. In protected Zaria districts the incidence



Map III.—Clearances in Katsina, Kano and Zaria Provinces. Figures show original 9.5 infection rate per cent. 1931-1933. Anchoed thetic-free area cross-hatched evacuated land dotted.

of disease is in no case above 0.5 per cent. In three neighbouring Katsina areas, where no dispensary treatment has been available, 1946 findings were —

1 Galadima. 1938 survey faulty in east of district only. No clearings. Average of 2.8 per cent. sleeping sickness in 1945-48, with high incidence, up to 18 per cent. in west of district.

2 Kankara and Dan Ja Original rates 11 and 7 per cent Clearings unorthodox, 1946 rate 0.9 per cent, but low rates in properly protected villages and high rates in those poorly or not protected

3 Kogo Original rate 9 per cent Clearings orthodox and efficient, 1946 rate 0.1 per cent

In future, an entomologist and six or more control officers may be engaged whole time on protective clearance work, and development officers may assist

#### AGGRESSIVE CLEARANCE—THE ANCHAU SETTLEMENT SCHEME

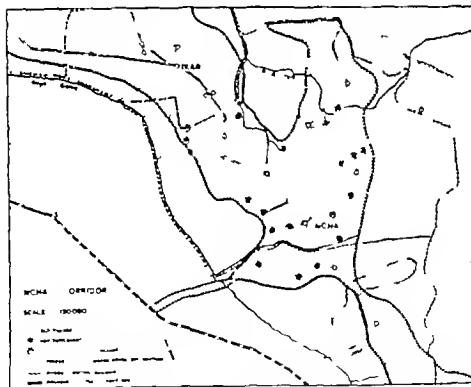
Colonial Development Fund assistance having ceased in March, 1947, maintenance of the settlement scheme is now to be gradually taken over by the Zaria native administration and by the Provincial Development Committee, on which all departments are represented, and will henceforth be under the same direction as other development schemes. Development, and especially rural development, is in the air these days. The question used to be asked why did the Sleeping Sickness Service undertake well-sinking, stock-rearing, agricultural work, etc., at Anchau? LESTER's answer was that, when he first put up his proposals to include rural development as an integral part of the scheme, everyone was agreed that rural development was urgently required, but no one wanted to be the first to start. The £95,000 grant, part of which was spent on Anchau, and which was regarded as a truly enormous sum, enabling us to do what no others could do, or ever hope to do, is nowadays almost chickenfeed. To criticisms of expense, one grew tired of insisting that Anchau was an experiment, the first of its kind, and that staff took months and years to train. Now, numbers of officers would not spend weary and costly months afoot doing topographical, vegetation and other surveys, one would not start until full air surveys were complete, and then probably go in with helicopters and bulldozers.

The Anchau tsetse-free corridor, linking two of the railway lines that diverge from Zaria, is some 65 miles long, over 600 square miles in extent and with a population of 50,000, slightly smaller than originally projected. It is tsetse-free. NASH's combination of partial and barrier clearings is effective. The annual re-slashing of the clearings is now a light task, less than 1 day's work per adult male. Corridor inhabitants still get sleeping sickness in their journeys away from home, 0.2 per cent were treated in 1946. Visitors to Anchau are perhaps sometimes bored when we stop at a river to show them the grass growth and lack of erosion in a barrier clearing (Fig 3, facing p. 462), or streams where the only remnants of dense thickets are well-spaced stately mahoganies, sometimes with patches of sugar-cane or irrigated market gardens.

NASH and his assistants spent their first 2 years in making the basic topographic, demographic, agricultural and economic surveys on which the population movements were based, and on which much of the success of the scheme rests. The problem was not one of moving primitive people wholesale into

new lands, but of reintegrating a whole social and economic complex, especially in Anchau District where the major moves were to be. The people from hamlets to be evacuated had to be dovetailed into the ancestral lands around their parent township not just into any convenient vacant land. There must be no over crowding and soil impoverishment. The new settlements had to be model villages with a richer life, based on permanent agriculture animal husbandry assured water supplies, higher standards of hygiene, variety of crops and fruits for consumption and for sale, and controlled forest management.

Map IV shows the centre of the corridor and Anchau District. Formerly in this district, 13,000 people occupied 977 square miles. By moving 3,700 of them from forty two hamlets scattered throughout 200 square miles in the wings of the district, the whole population now occupies 170 square miles, and most of the evacuated land is forest reserve. Before moving these people there had to be determined their land requirements and the agricultural possibilities of the patches of land available for them between existing villages. The



Map IV — Central part of Anchau Settlement Scheme showing settlements and principal old villages. Evacuated areas marked. Reserve in this part of the insect-free area there are over 30,000 people and 83 cement lined wells.

former was measured by detailed surveys of farmlands in established representative villages, for all purposes, including fallow, grazing and fuel, each individual required 4.3 acres. For land values a series of plant indicators was evolved, vegetation surveys made over 90 square miles, and trial plots on typical soil formations planted with staple crops. The vegetation surveys alone took five officers 5 months to complete. Possible village sites could then be chosen, trial shafts for water sunk, and the village elders brought to make their choice. The layout of the new villages, built under the direction of the Administrative Officers who superintended the moves, varies, but where possible compounds are grouped around a central well space 100 yards square. The unit compound of 100 feet square suits the average farmer. Between compounds or blocks of compounds are 100 feet spaces and all huts are at least 12 feet apart. Between and for 30 yards behind compounds tall crops are forbidden.

With the movement of villages, the congested slums of Old Anchau were cleared, some of its population and the district market being moved to a new town, Takalafiya. Part of Takalafiya is seen in Fig 4, a standard compound in the foreground, the district head's office, the mosque and courthouse in the background beyond the well space.

Once the new villages were completed, attention was for a time directed almost solely on their development. By 1942 this could be relaxed to allow gradual resumption of well-sinking, general development of the whole tsetse-free corridor and protective clearance outside the corridor. One of the greatest boons at Anchau was the general provision of wells and the gradual elimination of guineaworm, a standard well-top with rollers and basins is pictured in Fig 5. A steady drive was made to improve local products and to introduce variety into the diet by the distribution of special seed, supplied by the Agricultural Department, on the return-at-harvest system, by preaching simple methods of composting, by the introduction of tobacco, pigeon pea, and soya and the planting of thousands of fruit trees, by encouraging irrigated farming, sugar-cane growing and sugar-making, by teaching approved methods of hides and skins preparation and by arranging marketing facilities, and by preaching the low-crop belt. A programme of livestock improvement was undertaken. Together with the encouragement of mixed farming on the approved lines, small herds of cattle have been built up in some villages to make the peasants cattle-conscious, a wise preliminary to their acquiring expensive bullocks and ploughs, donkey herds in other villages provide manure and transport, nomadic Fulani cattlemen are attracted to the district for longer or shorter periods, sometimes for good, by the veterinary department's immunization camps and animal clinic. Pig-breeding has been pushed, and last year thirty-nine pig-keepers made £440 cash from the sale of weaners for fattening. Improved poultry and goats have been introduced. These benefits are shared not only by new but also by old villages, which are cleaned up as much as possible, given wells and have low crop belts and fuel reserves demarcated. What of the future of the scheme? For some years responsibility for all



FIG 8.—Livestock Improvement. The householder who owns the pigs and brood mare shown, is an old S.S. case and runs a demonstration mixed farm.

possible projects has been gradually handed over to the district head with his village headmen, and to the native administration forestry veterinary sanitary and agricultural assistants who have worked with us for years under the direction of their European provincial officers. In latter years regular visits have been made to all villages by a propaganda team, representative of all departments, who teach, preach, advise and report on all developments. This propaganda work should continue. The weakest link in the native administration chain is often the hamlet headman, but the Anchau district head has now extra assistants to maintain contact with the hamlets. In ten central villages little schools have been started. They teach reading writing, simple arithmetic, weights and measures, handicrafts and religion—the Koranic teacher who gets 7s. 6d. a month for his services, is shown on the right in Fig 7. From 250 to 300 children attend and about 200 adults, the latter irregularly Fig 8. Tuition is in Hausa, and soon the new Gaskiya Corporation should be producing in addition to a daily paper textbooks and pamphlets on rural subjects, which these pupils will be able to read for the instruction of their families.

The Sleeping Sickness Service will continue to work in the corridor—there are offices, stores, dispensaries, dispensary attendants school, and the maintenance of the clearings to keep us there—and will be represented on the committee controlling the scheme. At present a sleeping sickness control officer is in local charge later there may be a development officer. Funds are still required, some £1,800 a year and are to be provided partly by the service, partly by the native administration. This amount should decrease as items like pig breeding

and village cattle herds become self-supporting, and the main recurrent charges apart from native administration and technical staff, will be for village schools and maintenance of wells.

What of future schemes? Basic policy to control sleeping sickness is mass treatment, dispensary follow-up and clearance by communal effort. Very small concentration schemes may be required in many districts for remote hamlets whose man-power is too low for self-protection. These should be done by Provincial Development Committees. Large-scale concentration of population is not likely to be required solely to control sleeping sickness, but there are other advantages of concentration in sparsely populated areas for water supplies, education, roads, technical advice, disease control, and so on, are more easily and more cheaply provided. There will be other development schemes, primarily agricultural or veterinary, in which elimination of tsetse is essential. The Sleeping Sickness Service may not initiate new schemes, but will assist in schemes similar to the pioneer one at Anchau. There are also many densely populated areas in Kano and Katsina in which aggressive might well replace defensive clearance, without the necessity for movement of population.

In speaking of tsetse control, I have mentioned clearance only. We have not yet been able to try out SYME's method of hand-catching, which LESTER (1939), however, pointed out would be applicable only in limited areas in Nigeria. We hope that methods of eliminating fly other than destruction of timber, e.g., insecticide, will eventually be found. Our forestry friends would be most delighted if such hopes were realized.

## SUMMARY

1 Conditions conducive to a high degree of contact between man and riverine tsetse are common in Nigeria, especially in the Northern Provinces.

2 The occurrence of strains of *T. gambiense* naturally resistant to arsenicals demands the administration to all infective cases of antrypol or a similar drug. This has been the rule in Nigeria since 1934.

3 Since 1931, 3,202,295 people have been examined at primary surveys, and the average infection rate was 9.6 per cent. Incidence was high (10 to 20 per cent) in central provinces, lower (1 to 5 per cent) at the edges of the endemic area. In peripheral provinces, despite similar contact with fly, infection was sporadic or focal.

4 "Mild" trypanosomiasis may be associated with depopulation. If not so associated, such a condition is not necessarily stable. A toxic exacerbation of previously mild symptoms is a common cause of death.

5 Standard treatment is a combination of tryparsamide following, or simultaneously with, either antrypol or pentamidine, the latter combination being less toxic. Results of treatment, especially in survey cases, are good. Administrative arrangements to ensure early diagnosis and regularity of treatment are essential.



6. As a prophylactic pentamidine appears safer than, and superior to, antropol, and is being used in mining camps.

7. Mass treatment and dispensaries have reduced infection rates to about 1 per cent. in the highly infected central endemic areas. This incidence does not seriously affect the public health but there must be positive evidence that infection rates are not increasing and proper use must be made of the treatment facilities provided.

8. Permanent control means vector control. 500,000 people have been protected by defensive clearings, and another 50 000 live in the Anchan corridor which has been made tsetse-free by a combination of partial and barrier clearings. Movement or concentration of population is a last resort. At Anchan experimental concentration was associated with measures of rural development.

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## DISCUSSION

**Dr C Hollins** Dr McLETCHIE asked me to come along tonight and give him a little moral support but I do not think he needs any support, either moral or physical. He has said that he is merely the recorder of other people's work but that is far from being the case. Under Dr LESTER we were engaged in the mass treatment of sleeping sickness and trekked about the bush seeing thousands of people and finding hundreds of cases every day. It was all very easy because it was under direct European supervision. Dr McLETCHIE has had the major share of converting the mass treatment campaign into a dispensary system which I think can be said to work without any European supervision whatsoever. It is a measure of Dr McLETCHIE's success that the opportunities to inculcate the arts of native administration than is treatment centres. I think that is a real danger we have to face. Dr McLETCHIE, I hardly need say, is a first rate Hausa scholar and his knowledge of Hausa life and customs is probably greater than that of anyone else in Nigeria. There are one or two points in his paper which I think he can tell us more about. At the beginning he mentioned that in Southern Nigeria where there are 10 000 000 people there is almost no sleeping sickness and innumerable reserves. I think that is a point that needs explanation and possibly research. It seems very important. Is it possible that these people have a natural immunity? What would have happened if no sleeping sickness work had been done in Nigeria? He has given us a slight comparison between the effectiveness of mass treatment with dispensaries and mass treatment followed by no dispensaries in Katsina province. Could he give us a comparison between Katsina where there has been mass treatment only and Zaria where there has been mass treatment plus clearing? He says that the result of pentamidine prophylaxis will take years to assess but perhaps he has had some success? I know there are places where there are high infection rates and where they started getting figures nearly 2 years ago and there should be results from those. One other point I would like to touch on the question of the "mild" district. It is extremely interesting. The infection rates have been so much reduced that it is difficult to demonstrate them. A number of medical officers were sceptical and thought it was a waste of time, because the people seemed well. My own belief is that the mildness of the area depends on the state of the epidemic when you begin visits. If you visit early in the epidemic I think you find masses of mild cases. If you visit later on many of them will have recovered without treatment, many will have died and you will find the actual cases are those of obvious advanced sleeping sickness. That is the only way I can explain the difference you find in different areas but I should be interested to have Dr McLETCHIE's views.

Dr K W Todd I want to ask some rather elementary questions. First of all, will Dr McLAREN tell me whether the formol-serum reaction has come to anything? It was useful, perhaps chiefly as a negative test, when we used it in the Congo. Another point in diagnosis Dr McLAREN mentioned that a number of lumbar punctures were done but only by doctors. The Belgians have always been ahead of us in training medical assistants, and I remember one of the Belgian sanitary inspectors told me that he had done 110 lumbar punctures in a day!

Another point is halarsol as an aid to diagnosis. Some of us used it against sleeping sickness. I found that it had no apparent effect on the disease, but it cleared up yaws where they existed along with the sleeping sickness. One case I remember because I gave a long course of halarsol and suddenly found that he had masses of trypanosomes in his glands. Just when I decided to give it up his parents took him away. They brought him back in a dying condition, and he died after an injection of trypanamide. A veterinary surgeon from Nigeria said he found that halarsol in infected cattle produced flooding of the peripheral circulation with trypanosomes—presumably they were *T. brucei*. When I went to Sierra Leone in 1931 there was no sleeping sickness reported in my area: there was a little around Freetown. It is now very common in that corner near Liberia. At that time I was looking for clinical cases of sleeping sickness and one man was reported to me as a funny man. I asked how long he had been funny and was told it was about 3 months. So I examined him for trypanosomes and gave him halarsol and re-examined without result. But I found that my ward was rocking with laughter whenever the man was in it. I enquired and found he had been court jester for about 3 months. Hence the history! There is now a great deal of sleeping sickness there, as well as in Liberia, especially among children. Seventy per cent. of the school children at one station are reliably reported to have got it though its existence has been officially denied.

One other point relates to what is called the selective destruction of bush. I wish Dr STACEY MORRIS had been here to tell us about this because it appears to be of very great importance, and I do not think it is the same as partial destruction. It is the destruction of so-called pre-historic types of shrub. Dr MORRIS told us that in the north part of the Gold Coast he can train a boy to select the thirty or so different varieties that he wants destroyed and such clearing makes no essential difference to the forest. From the point of view of farming it makes no difference.

Pong Tamale, a great veterinary project of the Gold Coast government was saved by the same system of insect eradication. We are now coming to believe that we can have satisfactory vector control, and I believe Dr McLAREN has got it.

**Dr Willson Rao** I have little to contribute to the discussion but I am glad of the opportunity to pay a tribute to the lecturer and to the Nigerian Sleeping Sickness Service.

Dr McLetchum's lecture shows an almost complete abnegation of self but those who know the work he has done realize how big his contribution has been. To a marked degree he won the confidence of the population of Northern Nigeria and as a result has obtained a unique degree of co-operation. Scientific planning will not get us very far unless the human element is fully considered. During the war the Nigerian Sleeping Sickness Service had an unfortunate time as a result of repeated cutting of staff and plans had to be curtailed, altered or even abandoned at a moment's notice. Nevertheless the results now seen are evidence of what was achieved.

The Ancliam experiment was an extremely fine conception and one of the main lessons from this experiment is that no Service of Government can efficiently work by itself in a watertight compartment. The good results obtained are directly due to the co-operation between the various departments. We must look at the whole social and economic picture and on that basis we must work in all Colonial development. The Medical Department cannot stand on its own feet. The Sleeping Sickness Service as described by the lecturer expanded the dispensary system and we hope with full co-operation of the people to extend this work to eradication of other major public health diseases. It is largely on an expansion of this conception that the mobile units of the present development scheme are being built up.

That Nigeria realizes what is owed to the Sleeping Sickness Service is shown by the fact that the initial training of these mobile epidemic units is largely in the hands of Dr McLetchum.

**Dr A J Duggan** It is a totally unexpected privilege to be asked to say a few words. I am nevertheless anxious to endorse Dr Hollings's remarks on the pleasure it has been to work under him for 3½ years. There are many points in the lecture worthy of wiser comments than I can make and that is on the satisfied if I can make one contribution to this discussion and I have carried out three fairly large surveys within the last 18 months in various parts of Northern Nigeria and I think it is possible to correlate Dr McLetchum's belief that "a very large proportion of mild cases die from acute toxic exacerbation of the actual trypanosomiasis" with one of the conclusions that Dr Hollings defined in a recent number of the *Transactions*. He regards a 10 minutes reading of the blood sedimentation rate as being indicative of the patient's equilibrium to the parasitic invasion. In doing these surveys I came across, on the average, about one hundred adult male patients suffering from

trypanosomiads, and I noticed that the higher sedimentation rates occurred not in the more advanced cases with the higher cell count but in the people with relatively early nervous system involvements and cell counts ranging between 30 and 40 per c.mm. of C.S.F. If we incorporate Dr. HOLLINGS' proposition along that line then these are the people most likely to die from acute exacerbations of the toxæmia. I think this is the critical point in ordinary mild cases of trypanosomiads, when they either die of the toxæmic manifestation, or turn the corner. In the latter case their sedimentation rates begin to fall again—they have managed to regain a little biological equilibrium to the parasite and they live on, to become typical chronic cases, and die from intercurrent infection and inability to hold their own in the competitive life of the bush. One factor which may produce breakdown at the critical period is I think, work. Dr. HOLLINGS himself wrote about a case of a carrier of his, perfectly well apparently working for him, who died of an acute toxæmia within a few days of putting his load down after a long trek. This sort of breakdown can be brought about on a mass scale, by malnutrition after failure of crops, or an unusually heavy farming season causing a great deal of work among the adult male population. These people may die suddenly soon after being seen in apparent good health by lay officers touring in the bush who, when told that 5 or 10 per cent. of sleeping sickness of the mild type exists, may be somewhat sceptical because they do not see the classical cases. Further on questioning these patients with early nervous system involvement 60 per cent. admitted to having no symptom whatever. That is, 60 per cent. of people at the most critical stage of sleeping sickness would not in the ordinary way seek dispensary treatment. This may be one answer to the problem of what happens to these so-called "mild" cases of West African trypanosomiads.

Professor P. A. Buxton. I wish to say a word of appreciation of Dr. McLACHLAN's paper which I have enjoyed very much. One of the things we most need is expert summaries of large pieces of knowledge. It is extremely difficult to be up to date, and a paper like this presenting a broad view of a large subject by a man with first hand knowledge is of great value. The paper is also welcome because people who ought to know better often tell us that little is being done about sleeping sickness. I have come back from prolonged travelling in East Africa, realizing what a very great deal has been done by British administration on that side of the continent.

Here we have the story of a major conquest in West Africa. I have myself seen Anchau. A large number of people and cattle are living healthily there and this is one of the most remarkable things you can see in Africa in the whole range of medicine and public health. Moreover the work has been efficient and cheap. When you consider the figures you have to remember that Anchau was a first effort, and that much time and money was spent working

out methods which it will not be necessary to do another time. But even then they spent about £70,000 and have about 70 000 people with cattle in health on land which was previously under fly with a high sleeping sickness rate. I notice with some regret that Anchau, which is so very important, is being handed over to the Zaria Native Administration and Provincial Development Commission. I feel that Anchau should have continued to be administered by the people who produced it as a demonstration. I have very much enjoyed the paper and we shall value it in print.

**Dr C C Chesterman.** My tribute to this paper and to the work done is in the nature of a suggestion. A film should be made and shown in this country, in the United States and in Lagos to demonstrate the kind of thing being done for the common weal in the commonwealth. I would like to make one or two comments. One is about the presence of *G. morsitans* in the central area where there does not seem to be any sleeping sickness. It provides another argument against the mutability of animal strains. On the question of survival I note there is some little divergence between Dr McLetchie and Dr Hollins. I have never known a case in the Congo surviving for a 10 year period without treatment and I ponder what follow-up has been observed in this case? One remembers that in our own country the Public Health Service started as the response to a foul disease—cholera. Sleeping sickness in West Africa has a similar role with the proboscis of the tsetse fly stabbing the Service into activity. With regard to clearings they seem to me somehow to belong to the pre pentamidine era. I would like to reserve judgment as to the necessity for those extensive clearings which demand continuous care and great expense and which seem somehow rather precarious and dependent on the non-introduction of fly again. The fact that people have to go a long way for firewood means that they are tempted to enter infested regions and bring back infection. One wonders whether pentamidine will not provide a better solution than clearing operations. I would like to express my appreciation of the development of the dispensary system and my hope that this will be metamorphosed into general health centres dealing with all diseases. I would like to know what plan Dr McLetchie has for training a suitable staff.

**Professor G Macdonald.** May I say how much I have enjoyed listening to this paper, not only for its fascinating account of successful work in the control of trypanosomiasis but also for the description of the way in which general public health measures have been combined with the work of the veterinary, agricultural and educational services in Anchau, I feel that this alone can truly be called preventive medicine. Any one of the services itself can produce but little effect. In combination they can revolutionize

the medical and social picture in a very short time. I have recently seen in East Africa the beginnings of similar schemes, not intended primarily for the control of trypanosomiasis but including that in their work—the Sukumaland Resettlement Scheme in Tanganyika is an outstanding example. Its primary object is to prevent soil erosion by the proper distribution of population—a movement which involves the combined activities of all services. I should therefore, like to support Dr WILSON RAE's appreciation of the value of this type of work which can be as successful in peace as combined operations were in war.

**The President, Sir Philip Manson-Bahr.** Unless anybody else wishes to speak I should like to congratulate the speaker once more and to comment on one little point, that is, to cite some instances that were apparently cured by one injection of antypol. My mind goes back to a case I inherited at the end of World War No. 1. His name was Johns and he was infected on the Congo in 1916. Johns had been treated with every known drug in the whole pharmacopoeia. At the time he was being given salvarsan and what not. He lingered on until 1921 when he had more trypanosomes in his peripheral blood than ever. He had had daily counts for years and had been in hospital for 4 years. He had trypanosomes in his cerebrospinal fluid and was going down rapidly when I learned that Dr WEXROV at the Wellcome Bureau of Scientific Research had a drug called Bayer 205 with which he was injecting mice. It was just before Christmas Day 1921 and I went over to Dr WEXROV and asked for a gramme of this drug, and he gave it to me. That was injected intravenously into Johns and the man was cured and I believe that this one injection cured him. Mr Johns went to Lever Bros., Port Sunlight on Merseyside, and I think he is still alive. That was to me one of the greatest miracles I have ever seen in medicine.

**Dr McLetchie (in reply).** First of all I must thank Dr HOLLINS for the complimentary remarks he made. He has spent 7 years in one province, Benue, and he knows more about sleeping sickness in that area than anyone else.

As to his question about sleeping sickness in the Southern Provinces where tsetse fly is widespread the answer is that we do not know but we hope that the new Research Institute will make a point of finding out. We know that the riverine tsetse *G. palpalis* is very common in the Southern Provinces but it is not there purely riverine. It is commonly found in the forest away from the rivers. There is some contact with man, although possibly not so close and not so repeated as in the Northern Provinces. That is one possible explanation. Another is that there may be some degree of racial immunity. In one outbreak in the Cameroons it was thought that immunity for some reason or other had broken down. Another very serious outbreak in the Southern

Provinces was probably due to the introduction of fresh strains of trypanosomes from the Northern Provinces. As to a comparison of areas where there have been mass treatment and dispensaries with areas where there have been mass treatment and some clearings but no dispensaries it would be difficult to make any clear statement. In certain areas in Zaria where there have been treatment, dispensaries and clearings, the infection rates are lower than in other districts where there has been mass treatment followed by dispensary treatment but no clearings. There are also areas in Zaria that are not cleared but have a low incidence. In one district without clearings or dispensaries, the infection rate 2 years ago was less than 0.2 per cent. Why that should be so while neighbouring districts have had 1 or 2 per cent, we do not know.

Regarding the progress of pentamidine prophylaxis I have no figures here that I would care to quote, but we have not yet seen infections in one small area in Zaria, and we have now given thousands in another area, and it seems that pentamidine is a sound prophylactic.

Regarding the suggestion that one's view of the "mild" sleeping sickness depends on the stage of the epidemic at which one enters the area, I do not think that this is altogether the case. Even in the stages of the epidemic when acute and advanced cases may not be uncommon, the majority are still infected by the "mild" trypanosome. In most areas, especially in the north, the mild case of trypanosomiasis is still much the commonest. At a later stage there may be a higher proportion of advanced cases. In some areas in Benue Province the proportion of advanced cases goes up to about 50 per cent. That is probably due to selection. Strains that are sensitive to treatment die out while strains which do not respond well to treatment tend to persist.

The President mentioned a cure after one injection. There are cases which Dr. Hollins followed up 20 months after one injection of antrypol, and others I saw about a year after he had given them one injection of pentamidine. They appeared to be healthy and some were possibly cured, but we put them on to a full course of treatment. There are records of cure of sleeping sickness by one or two injections of many of the drugs that have been used at one time or another. They were given one or two injections, failed to come up for further treatment, and were found after a year or two to be perfectly healthy.

Dr. Todd asked whether we used the formol-gel test. We use it sometimes. It is of some value in sleeping sickness. In a patient who comes from an endemic area and who has suggestive symptoms one may not be able to find trypanosomes and may have no laboratory facilities. If the reaction is positive and the Ide or Kahn is negative the case must be treated for sleeping sickness. But the formol gel should be positive in 15 minutes. Regarding the question of reaction, Hope-Gill (1938) found that 93 per cent of sleeping sickness patients reacted positively.



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cases were positive in an hour and that many were positive in 15 minutes. One could use the much simpler red cell sedimentation rate as described by Dr. HOLLAND and give antypol. If, a month afterwards, the rate is approaching normal, the diagnosis is sleeping sickness. In other diseases the sedimentation will probably remain abnormal.

With regard to the work done by staff other than medical officers, we have had a few of our best African attendants trained to do lumbar punctures. They are not allowed to do them except in the presence of medical officers, or exceptionally when a medical officer has ordered a lumbar puncture to be done but cannot be present. I do not think we would consider allowing dispensary attendants to do lumbar punctures on all patients.

As to the use of halarol, the suggestion that it might be used to cause a flooding of the blood with trypanosomes is interesting. We have been using mapharsin, a similar drug as a control for cases on melarsen oxide, and had quite a high percentage of cures in early cases. I think it even cured more early cases than did melarsen oxide. We did not notice any flooding with trypanosomes after one or two injections. I think every case was soon negative.

Regarding the selective destruction of bush, Dr. NASH has seen the Gold Coast work recently and he is publishing a report. He is one of the few persons who have seen Dr. MORRIS at work and his own work at Anchau. They appear to describe completely different things but mean much the same that is, cutting down all undergrowth and thinning out large trees. Dr. MORRIS describes it in a very exact way whereas we simply say "Cut down undergrowth and thin our large trees."

Dr. WILSON RAE mentioned co-operation between the different departments. This is very important in a scheme like that at Anchau. Such co-operation should be easier in future because every province has now a Development Committee in which all departments are represented. Professor BURTON said he did not think it wise to hand over Anchau to the Native Administration and to such a committee. All departments are represented on the committee which will supervise Anchau. We shall be represented on that committee, and in practice we shall have an officer at Anchau or based at Anchau for many years to come. Senior officers will visit Anchau frequently and will bring to the attention of the Development Committee anything we think not satisfactory.

As to Dr. DUGGAN's remarks on the milder cases, I think he should continue such work. If he does he will produce some very interesting results. Referring to the sudden death, I recently read DUTTON and TOON's report (1902) on the first European case. It was that of the Captain of the Gambia river steamer. When he came home to Britain for the second time about 18 months after he was first diagnosed, he put on weight and became stronger. He was leading an open-air healthy life and appeared to be regaining health but all of a sudden he went down with an acute illness and died within 3

days That is typical of what seems to happen in Nigeria Dr DUGGAN's work correlates the sudden breakdown with the stage when the nervous system is being invaded

Professor Buxton spoke of the smallness of the expenditure £70,000 to £75,000 was spent on Anchau It amounts to 30s per head over 10 years, or 2s 6d per head each year If we could spend the same amount throughout Nigeria much could be done, and there would be a considerable improvement in public health I do not think this would be spending too much money

Dr CHESTERMAN said he had never seen survival after 10 years in the Congo, and he asked about the follow-up Dr HOLLINS's cases were seen over a period of 20 months, and then seemed well Through lack of staff the follow-up could not be continued, and it was decided to treat all cases Doctors HOLLINS and DUGGAN saw them all and considered some were probably cured We have not followed up cases any longer than this, but I have seen cases giving a perfectly clear history up to 8 years, and one up to 10 years In some of them symptoms had disappeared I have seen quite a number of cases with a history of 3 to 5 years They had very mild symptoms the cerebrospinal fluid was normal, or had a slightly increased cell count There is no question that a number of cases go on to cure The trouble is that one cannot tell whether this is happening Dr CHESTERMAN also queried the expense of clearing, spoke of the great work of maintenance that has to be done, and of the possibility of the re introduction of the fly At Anchau there is little possibility of that, the fly cannot spread upwards from the main rivers It can be introduced following cars or cattle, but such a fly must die There is nowhere it can live and breed The work of re slashing clearings occupies only a few days at most and in a few years time the clearings will not need any maintenance at all beyond inspection and, perhaps, a modest slashing every 4 years Elsewhere the short defensive clearings at danger points are not designed to eliminate the fly but to prevent its penetration into the middle of the clearings Dr NASH has noticed that tsetse seems so completely dependent on man or man's animals that when an 800 yards clearance is made the fly may die out from the uncleared part of the river at the edge of the clearing In densely populated country one could expand the cleared areas by linking up or by some method of partial clearing in between the ruthless clearings I would be more suspicious of an area where pentamidine prophylaxis had been done and the tsetse fly left I think it would be much easier for infection to come in there In Nigeria there is a constant coming and going of population In one of HARDING's papers he mentions a household where there had been a 50 per cent turnover in population in 1 year If you completely eliminate sleeping sickness by treatment but leave the tsetse fly people from distant provinces are bound to come in with sleeping sickness and start epidemics again

As regards the dispensaries becoming health centres the Sleeping Sickness Service does medical and health work. At Zaria each dispensary has two attendants one of whom is trained in health work. In one year when there was an epidemic our Service treated 5,000 cases of meningitis. That was the time when Dr. Hollins spent 6 weeks without sleeping twice in succession in the same place. As to the training of staff many of our old dispensary attendants were almost illiterate. Nowadays we get boys who are educated up to a reasonable standard but we find it almost impossible to get highly educated boys to go to the bush. They can get cushy jobs in the large towns. Training is limited by the educational standard but we are gradually raising the minimum standard. The difficulty seems to be that there is nothing suitable between the low level of the dispensary attendant and the much higher level of the Assistant Medical Officer. The ordinary nurse or dispenser with hospital training is not very successful in the bush. At present dispensary attendants are taught to use the microscope, not only for sleeping sickness, but for hook worm and other helminthic diseases, and they are all given some medical and health training. The whole problem of raising their standard depends on getting enough European officers to help in field work and in teaching.

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## COMMUNICATIONS.

### SLEEPING SICKNESS OF AN UNUSUAL TYPE IN SIERRA LEONE AND ITS ATTEMPTED CONTROL

BY

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\* We are greatly indebted to Dr C MICHIE, Colonial Medical Service, who in 1942 first discovered that an atypical strain of trypanosome had arisen in the Fuero area, and carried out some preliminary investigation. Grateful acknowledgement is due to Dr W P H LIGHTBODY, CBE, Director of Medical Services, Sierra Leone, for permission to publish this paper, and to Messrs May & Baker, who have supplied the pentamidine isethionate. Our thanks are also due to Dr E M LOURIE for obtaining for us the references dealing with observations on the cerebrospinal fluid of monkeys.

## I. INTRODUCTION

The present trial in mass prophylaxis was undertaken in an effort to control an epidemic of sleeping sickness which had defied mass diagnosis and treatment repeated three times in 4 years. No subsidiary means were employed such as control of population movement or anti-tsetse measures.

The district in which the experiment has been carried out comprises three chiefdoms—Soo, Gbanc hando and Mafindo (see map). It lies along the eastern border of Sierra Leone adjoining French Guines and covers approximately 200 square miles. It is roughly triangular in shape, being bounded to the north by the Kongotan Range, to the west by the Gori Hills, and to the east and south by the River Mela. The ground rises from 1 000 feet along this river to about 1,500 feet along the lower slopes of the hills.

The vegetation may be classed into three zones —

Zone 1—South of line joining Kurundo and River Yamblama there is forest and thick secondary tree growth following cultivation.

Zone 2—Between R. Yamblama and R. Mella the vegetation is transitional between Zones 1 and 3.

Zone 3—East of R. Mella and north and north-west of R. Morfi orchard bush and open savannah predominate with only thin fringe of trees and undergrowth along the banks of the streams.

The whole district is intricately supplied by streams all finally draining into the River Mela. There is tendency for them to be smaller in Zone 3 and for greater number to dry up in the dry season. In the heavy rains even the smallest, however, reach considerable proportions and throughout the district streams are never more than a quarter to half mile apart. Bordering vegetation is very dense in Zone 1 and minimal in Zone 3 while in Zone 2 both open stretches and overgrown banks may be seen in turn. The majority of streams are clear running.

The population of the whole district is approximately 14 000, with density ranging from 90 to 115 per square mile. Amongst the more highly infected population round Fucro with whom this report is chiefly concerned, the average density is about 70 per square mile. The majority of people live in small hamlets of under 100 persons. In all there are but thirty five villages with over 100 inhabitants and only six with more than 300. On the other hand there are 156 villages holding less than fifty persons. As result villages and hamlets are never more than 1 mile apart and considerable casual movement between them is constantly occurring.

The average annual rainfall for the district is between 90 and 100 inches. While the greatest falls occur in July to September the rainy season lasts from about March to November and an occasional storm may occur during the dry months of December to February. The mean annual temperature is about 75° F. the temperature ranging from minimum of about 50° F. on the coldest night in December or January to a maximum of about 90° F. on the hottest day in February or March. *Relative humidity* readings are not available for this district but some data are given in Table I which were obtained during the period mid-1945 to mid-1946. In Kailahun, some 30 miles to the south, where the climate is similar and thermographic tracings have been found to be very close. Figures for the daylight hours only have been given since they alone are important in relation to tsetse activity.

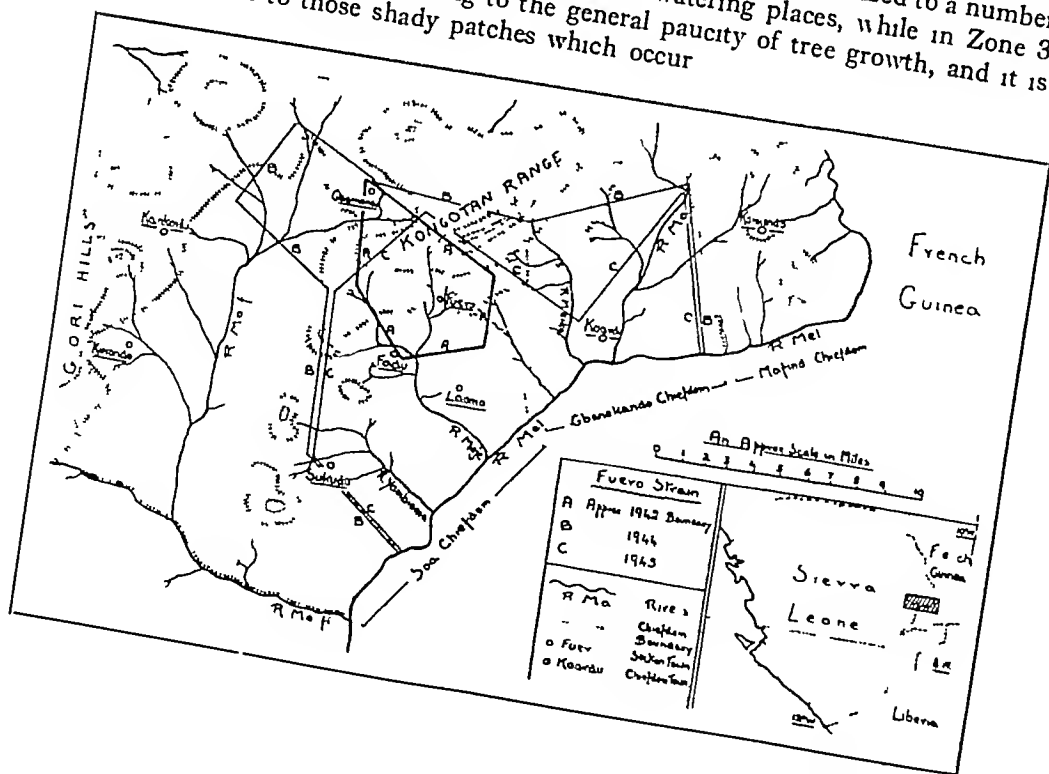
*Glossina palpalis* is prevalent throughout the district, where it is mainly confined to the immediate proximity of water though, particularly in the season of heavy rain, it may often be caught in the villages and on paths away from water. There are no striking seasonal changes in its overall density. To illustrate its approximate density some observations by one of us may be given.

TABLE I	
MEAN DAYLIGHT TEMPERATURE AND RELATIVE HUMIDITY AT KAILASHUN	
Month	
Jan.	60.0
Feb.	60.0
Mar.	60.0
Apr.	60.0
May	60.0
June	60.0
July	60.0
Aug.	60.0
Sept.	60.0
Oct.	60.0
Nov.	60.0
Dec.	60.0

Season	Months	Temperature ° F	Relative humidity %
Heavy rains	July to November	77	84
Dry season	December to February	79	57
Early rains	March to June	81	76

Along R. Meli a figure of about six flies per boy-hour (F B H) has been obtained except in the heavy rains when the figure was lower. A figure of two to six F B H was the average found in most of the main streams investigated. Certain isolated places have shown higher densities up to fifteen F B H, and these are almost entirely confined to points in the zone of transitional vegetation. Fly density is least in Zone 3, with its savannah vegetation—especially in Mafindo chiefdom, where the flies are localized to a few streams.

Man-fly contact, again, is greatest in the transitional zone. In Zone 1, owing to the general density of vegetation in this zone, it is localized to a number of more or less open patches at crossing or watering places, while in Zone 3 the converse is the case owing to the general paucity of tree growth, and it is here limited to those shady patches which occur





(iv) In proportion of cases trypanosomes were unusually burdensome in the blood.  
(v) The infection rate had slightly increased, indicating an enhanced transmissibility probably attributable to (iv).

All these features contrasted in greater or lesser degree with those of the usual run of cases in Sierra Leone, with those of the rest of the district under consideration, and even more significantly with those cases diagnosed in the same area the previous year. It was therefore apparent that a marked alteration in the local strain of trypanosome had taken place since the previous year and one had the good fortune to recognize the change when it affected an area of only about 14 square miles containing some 900 inhabitants. There was no reason to suppose that the tsetse situation had altered in any significant manner since the previous year and though their abundance and the close man-fly contact in the area might be expected to favour dissemination, it is difficult to postulate any way in which the vector might have altered the characteristics of the strain. It seemed more probable that a spontaneous mutation had arisen in the strain itself. It should be mentioned that no similar epidemic has been reported in the neighbouring cantons in French Guinea so that it is unlikely that the strain was introduced from there.

No obvious method of combating this new epidemic presented itself at the time, but all the inhabitants of the area were examined by taking careful blood films from every person in the hope of discovering the vast majority of cases and so of reducing the number of trypanosomes in circulation to a minimum. After this had been done and the additional cases found had been treated, no further work was possible in the area for over a year.

The third mass campaign took place in 1944 when a special effort was made to find all cases by again taking universal blood films besides performing gland puncture on any puncturable cervical gland however small. The system adopted was to commence at Fuero and work outwards until the borders of the area affected by the Fuero strain appeared to have been left well behind. Normal diagnosis was then reverted to, blood films being taken only where indicated by glandular enlargement or clinical symptoms. Line B—B on the map encloses the area affected by the Fuero type of case in 1944. It will be seen that a very serious spread had occurred, and this had taken place silently owing to the character of the disease. The incidence had also increased in Area B as a whole from 4 per cent. in 1942 to 7.8 per cent. in 1944. In Mafindo chiefdom to the east and in the western part of Soa to the west the new type had not penetrated, but the infection rate had increased slightly from 1 per cent. to 1.7 per cent. and from 2.4 per cent. to 3.5 per cent. respectively since 1942 (see Map, p. 483).

In the centre of the area round Fuero the characteristics of the new strain were even more marked. In Fuero itself the gland-blood positive ratio of fresh cases was 1/10, while over the rest of Area B it varied from 1/2 to 1/4. Again a high proportion of symptomless cases without enlarged glands but with numerous trypanosomes in thick blood films was noticed. A study was

made of the behaviour of the strain in the human host, and will be referred to later. All cases were treated with a course of antrypol and tryparsamide. It should be noted also that a sleeping sickness dispensary had been opened in early 1944 at Kainkordu on the edge of the district, and was well used. It was, however, realized that the dispensary alone would not be sufficient to check the disease owing to the large number of symptomless cases which appeared to exist and which favoured the silent spread of the epidemic. It was hoped, however, that the more intensive 1944 campaign would ensure finding the vast majority of cases so that the dispensary would then be able to keep the disease under control.

In 1945 a trial survey was made at Fuero using the universal blood film technique to assess the position. It revealed no reduction in the incidence of new cases, which amounted to 8.7 per cent of the population. The gland-blood positive ratio was 1:1, less abnormal than the previous year, but it was obvious that the situation had not improved and that something more would have to be attempted than repeated mass diagnosis and treatment supplemented by permanent dispensary facilities. Mass prophylaxis was decided upon, and was planned to embrace a wide region beyond that affected by the Fuero type of the disease. As a first step, another complete diagnosis of the whole district, followed by treatment of all the cases found, was undertaken. Again universal blood films were examined where there were indications that the Fuero type of disease existed. This revealed that in Area B the incidence of new cases remained at practically the same figure, but there were indications that the disease was to some extent tending to revert back to its more usual type, thus the gland-blood positive ratio was 1.5:1 and the proportion of cases without palpable glands or symptoms, but with positive blood films, was less than in the previous year. The number showing high trypanosome counts per field in thick blood films was also reduced. It was therefore more difficult to define the area now affected by the Fuero strain as it tended to merge with the normal type of disease. Nevertheless, it was clear that there had been a check in the spread of the 1944 area, and a definite reduction in the incidence of new cases was found in its eastern part where, in Gbance Kando, the figure had dropped from 7.8 per cent in 1944 to 4.1 per cent. The approximate boundaries of the area in which the Fuero type of disease was still present in 1945 is shown on the map by line C—C. It shows a slight regression over 1944. In those parts of the district lying outside Area C diagnosis showed on the whole a satisfactory reduction of incidence since 1944 except for a patch in the south-west corner, where a small epidemic had arisen. But the cases here were strikingly of the classical type with chains of large soft cervical glands, and in fact of the eighty-two cases there found all were diagnosed by gland puncture.

Thus the general position immediately prior to mass prophylaxis in the area affected by the Fuero strain was much the same as that found in 1944,

the highest count of eight to ten per field, woman of about 48 years of age, was symptomless and in very good clinical state. Heavy blood infestation was quite inconstant feature in any particular case observed over period, and patient who gave a high parasite count on one occasion might show scanty infection or have become negative at the next examination.

In order to study the frequency of negative phases in cases originally positive in blood or gland juice, seventy five such cases were subjected to weekly re-examinations over a period of 2 months in 1944 and seventeen to approximately monthly re-examinations over a period of 7 to 9 months in 1945. Table III shows the results of blood film examinations only in those cases originally blood positive.

TABLE III  
PERCENTAGE OF ORIGINALLY BLOOD POSITIVE CASES FOUND STILL POSITIVE ON  
RE-EXAMINATION INTERVALS. NO TREATMENT

Year.	Number of cases.	Percentage R.F. +						
		1	2	3	4	5	6	7 weeks later
1944	55	55	53	50	45	31	34	40%
1945	13	Percentage R.F. +						
		1		3	4	5	6	months later
		55	53	47	21	35	50	59

Repeated gland puncture on those cases originally found gland positive showed similar negative phases.

It should be remarked that in these re-examinations two thick blood films were made from each patient and every film was examined for 15 minutes before being declared negative. Similarly gland punctures were carried out on all cases with puncturable cervical glands, however small or hard, and the fresh juice examined for 15 minutes. If negative, second gland was sought and punctured if available.

Table III shows that rather less than one half the number of originally blood-positive cases is likely to be found positive on any one subsequent occasion. If gland juice and blood examinations are combined at the first and subsequent examinations the proportion is somewhat higher but even then the maximum percentage which gave positive results on any one re-examination was 62.6 per cent of the seventy five cases in 1944 and 66.6 per cent. of the seventeen cases in 1945. This finding was borne out in 1945 from a different angle. In July an examination of 287 persons in the vicinity of Fucro revealed twenty five new cases of sleeping sickness. Two months later these same people were re-examined and an additional nine cases, previously negative, were now found positive. Assuming that no new infections had

occurred in the interval, and that the same percentage of cases were missed at each examination, it can be calculated that only 64.4 per cent of the existing cases were diagnosed in July.

The mean of these figures obtained in three quite separate sets of observations is 65 per cent. This is an important figure since it implies that a maximum of only 65 per cent of existing cases can be expected to reveal themselves at a single examination by the method employed, in other words, at least 35 per cent escape detection. It is important also as a means of calculating how many existing, but missed, cases were unavoidably given prophylaxis as described in Part IV (page 500). Finally, this figure furnishes ample explanation of why previous attempts at mass diagnosis and treatment had failed to stem the epidemic. The additional factor, that most cases were symptomless, made the spread a silent one.

#### (iv) CEREBROSPINAL FLUID CHANGES

Table IV below gives the cell count distribution in 111 cases lumbar punctured before treatment in the area in 1945. For comparison, the distribution which had been obtained in another epidemic area not many miles away where the disease was of normal type, is also given.

TABLE IV

C S F CELL COUNT DISTRIBUTION IN CASES FOUND IN THE FUERO AREA (WITH FREQUENCY OF SYMPTOMS) COMPARED WITH DISTRIBUTION IN A NORMAL AREA

Area	Number of cases	Cell count per c mm			
		0-5	6-20	21-100	100+
Fuero	111	31.5	32.4	18.9	17.2
Kiss (normal)	Many hundred	14.3	11.1	38.1	47.3
		62.2	26.1	9.1	12.5

Thus in the Fuero epidemic there exists a much greater percentage with abnormal C S F and this is combined with a low proportion of symptoms. The most noteworthy feature of the table is the absence of symptoms in more than half the cases with a cell count of over 100.

Table V gives the changes in C S F cell count found in thirteen untreated cases re-examined by lumbar puncture after 7 to 9 months.

It will be seen that the count had increased in five cases, but had decreased in four and had remained normal in four, so that there was no general trend towards an increase over the period. This finding is rather puzzling in view of the high proportion of cases with raised C S F cell counts in the whole group lumbar punctured at diagnosis (Table IV).

TABLE V

CHANGES IN C.S.F. CELL COUNT AFTER 7-9 MONTHS IN 12 TREATED CASES.

Original C.S.F. cell count per c.mm.	Number of cases.	Count 7-9 months later		
		Normal (0-5)	Raised above original figure	Reduced but still above 5 per c.mm.
0-5 cells	6	4	2	0
6-20	4	3	2	0
21-100	1	0	1	0
100+	1	0	0	1

and raises the question at what stage of the disease the cerebrospinal fluid becomes affected. It seems very possible that in those cases in which C.S.F. changes occur the count rises soon after infection and thereafter fluctuates both up and down with almost equal frequency. It is interesting to note that three cases which originally had moderately raised count of six to twenty cells showed after 7 to 9 months return to normal. It suggests that normal C.S.F. cell count by itself cannot be taken as an indication of recent infection nor of lack of involvement of the C.N.S. or its membranes.

#### (v) SENSITIVITY TO TRYPARSAMIDE.

In 1944 in order to discover whether cases infected with the Fuero strain of trypanosome were readily amenable to treatment by tryparsamide, patients diagnosed in the Fuero area were treated by eight doses of this drug alone instead of by the routine combination of antrypol and tryparsamide. Among about ninety cases re-examined 1 to 5 months later only one case revealed trypanosomes 2 months after completing treatment. The remainder besides being negative to blood and gland examination showed no clinical signs of relapse. Again, a more direct small scale test for tryparsamide resistance was made in 1945 when ten cases all showing readily demonstrable trypanosomes in gland juice, blood film, or both received one dose of 0.5 grammes tryparsamide, and were re-examined about 48 hours later. In only one case could trypanosomes then be found. These observations made it clear that the infecting organism was very sensitive to tryparsamide. It is worth remarking also that treatment by the usual course of one dose of antrypol, followed by seven of tryparsamide, has not only been very effective therapeutically in the Fuero area but has also exerted a considerable influence in preventing reinfection. Thus the population examined in the region of Fuero in 1945 included 347 people who had been found infected previously. More than three-quarters of these had received this course (1 dose antrypol, 7 tryparsamide) between 1 month and 3 years before. The remainder had received three doses of antrypol and five of tryparsamide 4 years previously. Only four of them were now found infected—an infection rate of 1.1 per cent whereas in the remainder

of the population not previously treated the incidence of new cases was 5.4 per cent. It seemed probable that the intypol component of the treatment course had exerted a prolonged prophylactic action, the more so as not all of these four cases were necessarily reinfections, one in particular was thought to have been a relapse.

# (11) EQUILIBRIUM BETWEEN PARASITE AND HOST AND THE POSSIBILITY OF SPONTANEOUS CURE

Mention has already been made of the infrequency of symptoms and general good condition of many of the patients, also of the fluctuation in numbers of peripheral parasites and their frequent absence in blood films. In order to give a clearer picture of the relation or adjustment between parasite and host, cases may be roughly divided into three groups—(a) apparently stable equilibrium, (b) apparent mastery of the parasite by the host leading to spontaneous cure, (c) apparent mastery of the host by the parasite leading to eventual death. It is probable that the dividing line between these groups is not hard and fast, cases sometimes passing from one group to another.

## (a) Apparently stable equilibrium

Many symptomless cases in a state of apparent equilibrium revealed trypanosomes at longer or shorter intervals. For example—

<i>Teca Bondu</i> F 35		19 44	Gland +	Blood —	No symptoms
		15 9 44	,	—	(Scanty)
		23 9 44	—	—	
		2 10 44	—	—	
		12 10 44	+	—	
		29 10 44	—	—	
		5 11 44	—	—	
		13 11 44	+	—	
				—	Still no symptoms
<i>Sia Nepoh</i> F 46		18 7 45	No puncturable glands	Blood + (scanty)	
		18 9 45	C S F 3 cells per c mm	No symptoms	
		24 9 45	No glands	Blood —	
		14 11 45		—	
		20 12 45		—	
		20 1 46		—	
		22 2 46		+	(1 trypanosome per 15 fields)
		30 4 46		—	
				—	C S F 10 cells per c mm
				—	No symptoms
<i>Tamba Bouma</i> M 24		19 9 44	Gland —	Blood +	Clinical symptoms present
		18 10 45	+	—	(Disappeared before treatment could be given)
					C S F 4 cells per c mm
					Condition excellent

<i>Tambo Boivou. M 34</i>	14 11 45	Gland —	Blood +	(1 per 10 fields.)
	20 12 45	—	—	
	19 1 46	—	—	
	22 2 46	—	—	
	2 5 46	+	—	C.S.F. 3 cells per c.mm. General health good.

In this last case, there was amelioration in symptoms and clinical condition during the first 13 months, but trypanosomes were still to be found a further 6½ months later.

(b) *Apparent evolution towards spontaneous cure*

Nine cases may be cited as falling within this category out of seventy five, followed up by weekly blood, and where possible gland juice, examination for 2 months. These nine cases, all positive at the beginning did not reveal trypanosomes again during that period, and remained symptomless. A rather greater number were found positive on a single subsequent occasion only. The period of observation was too short to allow of any definite conclusion, but in the following two cases, trypanosomes were not seen again in a number of re-examinations covering 7 to 9 months (—

CASES.

<i>Finde Yanga. F 8</i>	18.7 45	Blood + (scanty)	C.S.F. 2 cells per c.mm.	No puncturable glands. Monkey inoculated with blood revealed trypanosomes after 27 days incubation.
	18.9 45	Small gland —	Blood —	
	28 9 45	No puncturable gland	—	
	23 10 45	—	—	
	14 11 45	—	—	
	20 12 45	Small gland —	—	
	20 1 46	—	—	
	29 4 46	Blood —	C.S.F. 2 cells per mm.	General condition excellent.
<i>Sahr Dadi. M 6</i>	19 10 45	Blood (scanty).	C.S.F. 1 cell per c.mm.	Numerous small cervical glands all negative.
	23 10 45	Blood —	—	
	14 11 45	—	—	
	20 12 45	—	—	Multiple small glands throughout.
	20 1 46	—	—	
	22 2 46	—	—	
	12 5 46	—	C.S.F. 1 cell per c.mm.	General condition good.

The period of observation though longer is still not long enough to conclude with any certainty that spontaneous cure has taken place, for which purpose 3 or 4 years would be required. One can only say that actual experience of such cases makes this conclusion difficult to resist. It must, however be admitted that a further four cases were negative to examination from 5 to 7 months between successive positive results.

The case of Finda Yusufu recalls one described by BARLOVATZ (1933) who was discharged 4 years after first diagnosis of sleeping sickness, in apparently good health and without any signs suggesting trypanosomiasis, no trypanosomes having been seen for 10½ months in triple centrifugized blood specimens or C.S.I., and C.S.I. cell counts and albumin having remained normal. She had received no treatment throughout. While SICÉ (1937) considers spontaneous cure to be a rare event in human trypanosomiasis, YONKE (1921) collected from the literature up to that date records of a number of untreated cases who were found to be in good health and apparently free from infection from 3 to 8 years after diagnosis and HARDING (1940) adduced indirect evidence derived from mortality rates that in one district of Northern Nigeria the disease was not fatal at the time of investigation.

(c) *Apparent evolution towards victory of the parasite*

Two cases may be cited as falling within this category.

		CASES			
<i>Fayia Juni</i>	<i>M 21</i>	19 44	Gland	Blood	No symptoms
		15 9 44		—	—
		23 9 44	—	—	—
		2 10 44	—	—	Headache and pyrexia
		12 10 44	—	—	Steady deterioration
		29 10 44	—	—	—
		5 11 44	—	—	—
		13 11 44	—	—	Very advanced clinical symptoms. Unable to walk. Soporific. Rapid emaciation.
(This man improved very rapidly with treatment without which he would undoubtedly soon have died.)					
<i>Kumba Doma</i>	<i>I 40</i>	19 10 44	Gland	Blood	(scanty) C.S.I. 28 cells per cmm. No symptoms
		14 11 45	—	—	—
		20 12 45	—	—	—
		20 1 46	—	—	—
		2 5 46			(6 trypanosomes per field) C.S.I. 99 cells per cmm. Deterioration in general condition. Pyrexia and general malaise.

These two cases serve to indicate that in a proportion of individuals the disease does progress steadily and may be expected to lead to eventual death of the host in the absence of treatment. The first case also shows that progression may occur without the reappearance of peripheral trypanosomes. In this patient they were not seen in the last six examinations spaced over a period of 7 weeks.

It is difficult to apportion the cases actually met with to these three categories since evolution is often very slow, but it seems fairly clear that the majority belong to category (b), in which the parasite lives at any rate for long periods in a state of equilibrium or commensalism with the host. It is reasonably certain that relatively few of the cases progress at all rapidly towards death since there is nowhere any sign of depopulation and the censuses which have



been carried out from year to year show no decrease in numbers. Moreover very few cases are met with in a clinically advanced condition. It should be remarked that in the Fuero area between one-quarter and one-third of the population have been found infected at one time or another and a further proportion will have escaped diagnosis. As there have been gaps of a year or more since the epidemic arose during which no treatment was available many deaths might have been expected to occur had the course of the disease been rapid in more than a small proportion of cases. It is very possible that the cases in category (b) shift towards category (a) when conditions are good and food plentiful, but may shift towards (c) in the poorer classes in bad seasons of food shortage.

The whole picture suggests that this particular race of man and this particular strain of *Trypanosoma gambiense* exist in a state of generally satisfactory adaptation to one another recalling the adaptation between game animals and their trypanosomes. Or to take another example, that between cattle and the less virulent strains of *T. congolense*. One of us was once concerned in an experiment with Zebu cattle infected with *T. congolense* most of the beasts harboured their parasites for long periods without much deterioration in condition some appeared eventually to rid themselves of the parasite completely some died. It is well known that infected cattle may remain perfectly well for long periods but often break down when fodder is scarce or they are put to hard work at the plough.

It would, however be entirely unsafe to assume that the Fuero epidemic if left to itself, would not change its present by harmless character. There are already some indications of a tendency towards the more normal type.

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Blood from five patients  
Cercarial monkey All  
The incubation period and  
infected animals is given

It is of interest to note the  
period, and that the animals  
was inoculated from an acutely ill  
known to have been only very  
inoculated was women of rather  
two years but normal C.S.F. cell  
mentioned in Section A, p. 494 who  
cell count and remained in error  
a period of 9 months observation  
cell count was still normal and she had  
patient and the monkey infected from  
this connection it may be recalled that

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of spontaneous cure in his monkeys C 54 was inoculated from a baby of about 6 months old whose general condition was so poor that it was considered inadvisable to keep him under observation and he was treated at once

Puncture of glands in the groin and axillae of three of these animals was also carried out Small glands were present before inoculation, and they did not enlarge markedly after infection had occurred In C 53 gland puncture was first positive 10 days after the first appearance of trypanosomes in the blood and remained positive for a fortnight, after which all subsequent punctures

TABLE VI

Monkey	Incubation period	Subsequent course
H 1	8 days	Blood remained heavily infected and animal lost condition until death from trypanosomiasis 2 months later
C 53	11 days	Blood rapidly became heavily infected thereafter trypanosomes became fewer but, except for an occasional negative phase, remained fairly constantly present usually at about 1 or 2 per field of stained thick film General condition has remained good and C S F cell count has not risen although infection still persists 17 months after inoculation
C 86	28 days	Trypanosomes became fairly numerous in the course of about a fortnight after their first appearance but thereafter declined in number and disappeared for good a fortnight later The animal has now been blood-negative and in good condition for 17 months
C 54	47 days	Trypanosomes remained rather scanty in the blood for 15 days and then disappeared The monkey, a young weakly animal, died of unrelated causes 5 weeks later without any reappearance of blood trypanosomes

carried out over a period of 3 months were negative In C 86 gland puncture was positive on two consecutive occasions only, the first occasion being 9 days after the appearance of blood trypanosomes C 54 never became gland positive Thus gland infection, when it occurred, appeared to be only a brief phase In the patients from whom these three monkeys were infected, cervical glands were either absent or too small and hard to be puncturable

Attempts were made to isolate the normal Sierra Leone strain of trypanosome for comparison, and for this purpose three *Cercopithecus* monkeys were inoculated with blood from patients living outside the Fuero area, but all failed to take the infection This in itself was instructive, as showing the comparatively high infectivity for monkeys of the Fuero strain

C.S.F. cell counts were carried out on a number of both clean and infected monkeys in order first, to try to establish the limits of the normal and then to discover any evidence of abnormality in the infected animals. The monkeys used have been of three species, thought to be *Cercopithecus callitrichus*, *C. nictitans* and a *Cercopithecus*—the sooty mangabey. The results are summarized below—

#### Clean Monkeys

First *C. callitrichus* 45 cells per c.mm. by lumbar puncture  
 Second ditto 17.11.45 8.5 cells 9.1.46 32 cells; 11.10.46 34 cells.  
 A *C. nictitans* gave on two occasions 8 and 10 cells respectively  
 A mangabey gave on three occasions 1, 4 and 3 cells.  
 A second mangabey gave the following counts on lumbar puncture 20.8.45 33 cells;  
 16.9.45 17 cells; 20.11.45 10 cells 14.12.45 14 cells.  
 On sub-occipital puncture this animal gave count of 80 cells.

#### Infected Monkeys.

*C. callitrichus* No. C 83 Inoculated 22.7.45 blood showed trypanosomes from  
 3.8.45 onwards. 20.8.45 17 cells 27.8.45, 23 cells 16.9.45 26 cells 20.11.45  
 14 cells 14.12.45 21 cells 9.1.46 18 cells 23.8.46 12 cells 11.10.46 27 cells.  
*C. callitrichus* No. C 66 Inoculated 22.7.45 blood showed trypanosomes from  
 18.8.45 to 16.9.45 20.8.45 19 cells 16.9.45 82 cells 17.11.45 9.5 cells 13.12.45  
 16.5 cells 9.1.46 22 cells 11.10.46 49 cells  
*C. callitrichus* No. C 54 Inoculated 22.7.45 blood showed trypanosomes from  
 7.9.45 to 21.9.45 4.10.45 5 cells.

Lumbar puncture thus provided no evidence of C.N.S. involvement in the infected animals.

These counts have been given in some detail because they contrast with the figures reported for normal monkeys by other observers. Thus in none of the clean monkeys examined by VAN HOOFF (1927-28), MOLLARET (1934), and COMBOY (1938), was a cell count of over 10 per c.mm. obtained. In some of our animals, both infected and uninfected, a proportion of the spinal fluids exhibited, in addition to lymphocytes, large cells with lobulated or multiple nuclei, and occasionally cells containing what appeared to be granules of brown pigment were seen. We can offer no explanation for these findings. The possibility of an incidental infection with a virus causing encephalitis occurred to us, but this explanation seems unlikely in view of the facts that the animals were of three different species collected from different parts of the country and that the counts sometimes remained high or fluctuated up and down over many months in the same animal. Whatever the explanation, our observations indicate that caution should be exercised in interpreting C.S.F. changes in monkeys used for trypanosomal study.

#### GUINEAPIGS.

Guineapigs proved more refractory than monkeys to infection by blood inoculation direct from man, and of four pigs inoculated only one became infected. However by comparison, at least a dozen attempts to infect guinea

pigs from patients infected with the normal Sierra Leone strain in the past have met with not a single success

On the other hand, subinoculation from Monkey C 53 into guineapigs was readily successful, and the infections resulting from this passage, and from subinoculation from pig to pig, usually ran a nearly uninterrupted course with few or no remissions until the blood became swarming. The period which elapsed between first appearance of the trypanosomes and the time they swarmed varied between 6 and 12 weeks. These pigs were used for *in vivo* tests of the resistance of the strain to tryparsamide and to normal human serum at a time when blood trypanosomes were numerous and steadily increasing.

#### *Resistance to Normal Human Serum*

Two c.c. of freshly collected human serum injected intraperitoneally into a pig of the second passage from man failed to influence the number of trypanosomes in the blood. A similar dose given to a pig of the fourth passage more than a year after isolation of the strain from man also had no observable effect. By comparison with this finding one of us, using a Nigerian human strain which exhibited some *rhodesiense*-like properties, had previously found this dose of serum sufficient to banish trypanosomes for several days in guineapigs a few weeks after isolation of the strain.

#### *Resistance to Tryparsamide*

A dose of 0.015 gramme/kg of tryparsamide given to a pig of the second passage caused trypanosomes to disappear completely for 12 days. A dose of 0.03 gramme/kg given to another pig of the second passage banished them for nearly 3 months.

Opportunity was also taken to give antrypol and pentamidine to infected guineapigs in doses proportional by body weight to those which had been given to infected humans (see Part IV, page 500), for comparison with the latter. The results are summarized below —

#### *Antrypol*

- |             |                                |  |   |
|-------------|--------------------------------|--|---|
| Guineapig 1 | 18.1.46, trypanosomes swarming | Antrypol 0.02 gramme/kg injected intraperitoneally | Trypanosomes disappeared and did not reappear until 20.4.46, <i>i.e.</i> , 3 months later |
| Guineapig 2 | 13.8.46, trypanosomes numerous | Antrypol given 0.02 gramme/kg                      | Trypanosomes disappeared, reappeared 22.11.46, <i>i.e.</i> , 14 weeks later               |

#### *Pentamidine*

- |             |  |  |  |
|-------------|--|--|--|
| Guineapig 3 | 19.3.46, trypanosomes swarming                   | Pentamidine isethionate given 3 mg/kg  | Trypanosomes disappeared and have not yet reappeared 7 months later, indicating presumptive cure |
| Guineapig 4 | 27.5.46, pentamidine hydrochloride given 2 mg/kg | Trypanosomes disappeared and reappeared on 9.8.46, <i>i.e.</i> , after 2½ months |  |

When trypanosomes reappeared after suppression by these drugs they behaved as they had done in the original infection and did not again disappear from the blood but tended to a steady increase.

These rather limited animal observations indicate that the Fuero strain is resistant to normal human serum but sensitive to arsenicals, suggesting that an atypical form of *T. gambiense* rather than *T. rhodesiense* has arisen in the

area. They also show that antypol and pentamidine in small doses exert a prolonged suppressive effect on the strain.

#### SUMMARY OF CHARACTERISTICS OF THE INFECTION

1. Paucity of symptoms.
2. Infrequency of cervical adenitis.
3. Occurrence of unusually heavy blood infestation in a proportion of cases.
4. Wide fluctuation down to zero in numbers of trypanosomes in the peripheral circulation over a period in the same individual leading to great difficulty in diagnosis.
5. High proportion of cases exhibiting altered C.S.F. cell counts.
6. High sensitivity of the strain to trypanamide in man and guinea-pigs.
7. Resistance *in vivo* of the strain to normal human serum.
8. High transmissibility.
9. Equilibrium between man and parasite with apparent tendency to spontaneous cure in certain cases.

#### IV. METHODS AND RATIONALE OF PROPHYLAXIS.

The area in which mass prophylaxis has been employed covers the whole of that shown on the map page 483 *i.e.*, Soa, Gbanc Kando and Alafindo chiefdoms, together with part of another chiefdom lying immediately to the north of these. The population thus dealt with numbered about 15,500. Beyond the confines of this area where it adjoins other parts of Sierra Leone, the sleeping sickness rate nowhere substantially exceeded 1 per cent. and in most parts was much less. On the French Guinea side the incidence of new cases in 1945 was 1.8 per cent. The intention in dealing with this block of country was not only to make it large enough to ensure that there should be no foci of high infection beyond its borders from which re-infiltration of the disease might readily occur but also to surround the area affected by the Fucro strain (except where it adjoins French Guinea on one side), with a broad band of protected communities. In this way it was hoped largely to isolate a part of this area for intensive study unaffected by outside influences. This report deals mainly with the findings in the central part referred to henceforth as the Fucro experimental area. It is hoped to publish an account of the ultimate results in the whole area at a later date.

The area selected for intensive study corresponds with that portion of Area C shown on the map which lies within Soa chiefdom, less the southernmost tip. It contains approximately 2,500 people. The objects aimed at in the work in this area were to establish —

- (i) Relative value of antypol and pentamidine as prophylactic agents.
- (ii) Dosage necessary to prevent infection for long periods—up to 6 months if possible at the end of which time it might be expected that all or nearly all, the flies already infected before prophylaxis would have died out.

(iii) Whether a single dose sufficiently high to provide such a protection could be tolerated by large numbers. If so, this would make mass prophylaxis a speedy and simple matter in future work.

(iv) Whether antipryol or pentamidine given only in prophylactic dosage would suppress the parasites in persons already infected with the local strain of trypanosome and if so, to what degree and for how long.

The last consideration became of practical importance because of the unusual nature of the epidemic. It will be remembered from a previous section that at least 35 per cent. of proved cases were negative to blood and gland juice examination on any one occasion, and for this reason it was inevitable that many infected persons would be missed and would receive the prophylactic dosage designed for healthy people. A number of proved cases were therefore deliberately given a prophylactic dosage only of one or other drug, and were then watched so as to provide a basis for assessing at the end of the experiment how many of the cases then found positive in the general population were actually new infections and how many were missed cases already infected before prophylaxis commenced. Only after making allowance for such cases would it be possible to assess the true prophylactic value of the drugs employed.

In connection with the duration of prophylactic protection, it should be recorded that the important correlative factor of the longevity of *G. palpalis* in the climatic conditions found in the area is unknown to us at present. But for the interest of others who may possess the required knowledge Table VII is included. This records some relevant data on temperature and humidity obtained from readings of a thermohygrograph run during the last week of each month at Karitumu, some 30 miles to the south of Luero, where climatic conditions do not differ very appreciably. Since prophylaxis commenced in November the readings given are for the following 6 months. The time of day at which maximum temperature is reached corresponds very closely with the time of minimum relative humidity.

TABLE VII  
TEMPERATURE AND RELATIVE HUMIDITY AT KARITUMU

Month	Temperature °C				Relative humidity per cent			
	Mean day	Mean night	Mean 24 hrs	Mean max	Mean day	Mean night	Mean 24 hrs	Mean min
December 1941	75	61	68	81	73	89	83	74
January, 1942	78	64	71	86	49	84	73	71
February, "	83	73	78	92	61	87	76	49
March, "	83	72	77	88	73	91	81	60
April, "	82	71	76	89	77	88	81	61
May, "	83	72	76	86	78	91	81	61
Whole period	80	71	76	88	71	87	77	61

#### PROPHYLAXIS IN EXPERIMENTAL AREA

At the outbreak of the population of the area was small, and it was therefore possible that all the inhabitants could be observed and examined for examination. For the whole population was then

carefully examined by making stained blood films from every person and by in addition obtaining gland juice from all subjects with puncturable cervical glands. C.S.F. cell counts were performed on nearly all cases thus diagnosed. Some of these cases were given full course of treatment; others were left untreated for observation in the manner already recorded in previous section; others again were given antrypol or pentamidine in prophylactic dosage only.

Of the remaining population in whom no trypanosomes or clinical evidence of sleeping sickness could be found 519 were set aside as controls and given no drug, while the remainder numbering 1 785 were given prophylactic injections of antrypol or of pentamidine in the dosage to be described in Part V.

It is important to describe here the manner in which the controls were selected. In most field experiments in prophylaxis, e.g. in that described by VAN HOOFF *et al.* (1946), the observer has selected every second or third person presenting himself or adopted some similar method of random selection whereby the controls are scattered through every village in which prophylaxis is to be tested. No doubt this is the best method of studying the duration of protection in the individual but we had an additional purpose in mind, viz. to estimate the success of prophylaxis when applied to whole communities. In the present study the population which received prophylaxis comprised whole villages, while other villages were selected as controls and none of their inhabitants received any drug. The control villages were selected as being representative particularly in respect of the infection rate found at the beginning of the trial (and hence presumably of liability to fresh infection), and were scattered all over the area. By using this method it was also expected that a better indication would be obtained of what the incidence of new cases in the area might have been during the period of observation if no prophylaxis had been applied. When some subjects are selected for prophylaxis, and others to serve as controls from the same village, the chances of infection among the controls are less than hitherto, since the prophylactically treated inhabitants of the village are no longer able to infect tsetse in the neighbourhood; in consequence the frequency of fresh infections will fall in the controls, in the same way as vaccination against smallpox of three-quarters of the members of a community may serve also to protect to a large degree the remaining one-quarter.

Finally a prophylactic trial with the same drugs was carried out for comparison in two other small areas affected by the normal Sierra Leone type of sleeping sickness.

## V RESULTS OF PROPHYLAXIS

### A. SUPPRESSIVE EFFECT OF ANTRYPOL AND PENTAMIDINE ON CASES ALREADY INFECTED.

These cases were given a similar variety of dosage to that employed for the purpose of prophylaxis in the population at large, and were re-examined monthly for the following 7 months, after which the experiment ended and all cases received a full curative course of treatment. Table VIII shows the numbers observed and the dosages employed.

TABLE VIII  
INFECTED CASES RECEIVING VARIED PROPHYLACTIC DOSAGE

Group	Drug	Dosage	Number treated	Not traced	Died	Observed 7/12
1	Antrypol	1 x 1 gramme	26	4	0	22
2	Pentamidine isethionate	1 x 100 mg	17	1	0	16
3	"	2 x 150 mg	24	1	0	23
4	"	375 mg in 2 inj	16	2	1	13
5	"	500 mg in 3 inj	22	1	2	19
	Total	—	105	9	3	93

The dosage stated is that given to persons of more than 100 lb body weight. Children received doses bearing the same relation to the full dose as did their body weight to 100 lb. Where two or three doses were given, they were administered at weekly intervals.

The three deaths occurred from unassociated disease.

Table IX shows the percentage of cases in which trypanosomes were revealed at each monthly examination after the administration of the drug. On every occasion two thick blood films were prepared from every case and each was scrutinized for 10 to 15 minutes, two punctures were made and examined whenever glands were present. In this table Groups 2 and 3 and Groups 4 and 5 of Table VIII have been combined.

TABLE IX  
PERCENTAGE OF INFECTED CASES REVEALING PERIPHERAL TRYPANOSOMES AT MONTHLY EXAMINATIONS AFTER PROPHYLACTIC DOSAGE

Drug	Total dosage	Percentage positive at							Percentage cases relapsing during 7/12
		1/12	2/12	3/12	4/12	5/12	6/12	7/12	
Antrypol Pentamidine isethionate	1 gramme	0.0	6.2	7.7	8.3	8.3	Not examined	22.7	27.2
	100-300 mg	4.2	10.5	7.9	11.1	12.1		10.0	35.0
	375-500 mg	0.0	5.7	0.0	0.0	0.0		3.1	9.4
All cases		1.1	7.9	5.1	6.7	8.1	—	10.8	24.7

Three points about these results are noteworthy. First, different cases were positive on different occasions, and for this reason the percentage of cases which revealed trypanosomes at some time during the period (last column)



is considerably higher than the percentage which revealed them at any one examination.

Secondly there is very little tendency for an increase to occur in the number revealing trypanosomes between the 2nd and 7th month. It might have been expected, as indeed was found to be the case in infected guinea-pigs which had received similar dosages reckoned in grammes per kg. that there would be complete suppression of trypanosomes for a considerable time representing the period during which the drug remained in trypanocidal concentration in the blood, after which parasites would reappear with the same regularity as in untreated cases. But with one doubtful exception in the antrypol group in which a jump to 22.7 per cent. of peripheral relapses occurred at the 7th month, this was not the case. In fact, it not infrequently happened that a case would show trypanosomes within 1 or 2 months after injection, but most or even all of the subsequent examinations would prove negative. This occurred after all drugs and dosages. An interesting illustrative case is that of a patient who revealed trypanosomes as early as 3 weeks after 100 mg. pentamidine, but only in one of the subsequent 7 monthly examinations.

Thirdly the percentage of cases relapsing during 7 months after the lower dosages of pentamidine is not significantly different from the percentage relapsing after antrypol but after the higher dosages of pentamidine the percentage is much lower. The same feature is apparent when the percentages of positive results in the total examinations carried out in each drug group are compared. In actual figures, after 100 to 300 mg. pentamidine, 7.6 per cent. of 262 subsequent examinations proved positive, while after 375 to 500 mg. pentamidine only 1.8 per cent. of 185 examinations proved positive—a difference which is statistically significant. This result might be explained on the basis of a more effective suppression of trypanosomes by the higher dosage of pentamidine and/or on the supposition that 375 to 500 mg. was sufficient to produce actual cure in those cases which were in an early stage (pentamidine being well known to be ineffective in the majority of advanced cases). To elucidate this point, Table X has been constructed, in which cases are classified both by drug received and by initial C.S.F. cell count.

The salient features brought out by this table are (a) in Group III the relapse rate is similar in cases with an initially normal and an initially abnormal C.S.F. cell count—this finding favours suppression rather than cure as the cause of the comparatively low relapse rate in this group. (b) in cases with an initially abnormal C.S.F., the percentage of relapses in Group III is about two-fifths of that in Groups I and II combined, whereas in cases with an initially normal C.S.F. count the proportion is only about one in seven—this would seem to indicate that some cures have occurred in Group III. On general grounds some cures would be expected since a dosage of 375 to 500 mg. approaches that which has been found curative in early cases by a number of workers. The cases in our series are not sufficiently numerous to justify definite conclusions, and a classification by cell counts of below and above five

is not entirely satisfactory since pentamidine is capable of curing some cases with slightly raised counts as well as those in whom the count is completely normal, but the findings suggest that the higher dosage of pentamidine has been more effective than the lower in causing suppression of peripheral trypanosomes, and in addition has produced some cures

TABLE X  
PERIPHERAL RELAPSES OVER 7/12 CLASSIFIED BY INITIAL C S F CELL COUNT

Group	Drug	Dosage	Initial C S F cell count 0-5 cells			Initial C S F cell count 6 cells		
			Cases	Relapses	Relapses per cent	Cases	Relapses	Relapses per cent
I	Antrypol	1 gramme	3	1	33.3	20	6	30.0
II	Pentamidine isethionate	100-300 Mg	13	8	61.5	26	6	23.1
I and II combined			16	9	56.3	46	12	26.1
III	Pentamidine isethionate	375-500 Mg	13	1	7.5	19	2	10.5

In the course of this study two cases of unusual interest were encountered which merit brief description —

Male of about 45. At original diagnosis blood positive (scanty trypanosomes), C S F 186 cells per c mm. Given pentamidine isethionate 100 mg. Seven months later blood positive (1 per 3 fields), C S F 2 cells.

Male of about 20. Gland juice positive, C S F 47 cells per c mm. Given antrypol 1 gramme. Seven months later blood positive (scanty), C S F 2 cells.

That these patients' cell counts should have returned completely to normal despite the persistence of trypanosomes is remarkable for its own sake, and important as another indication that a normal cell count does not necessarily demonstrate recent infection in this area, nor yet exclude past or present involvement of the C N S or its membranes. On final examination, both these patients were free from symptoms and in good physical condition. Since evidence has already been given in Part III, A, (vi), page 493, which strongly suggests that trypanosomes may disappear permanently without treatment, in some cases with a normal cerebrospinal fluid, it appears very possible that the same may eventually occur in cases such as the above in which the cell count, though originally high, has returned completely to normal. In fact, these two cases may be in process of spontaneous cure though trypanosomes were still present

on final examination. (The small prophylactic dosage each received was quite inadequate to influence C.S.F. involvement.) This possibility is of considerable interest since, although a good deal of evidence has accumulated in the literature to show that spontaneous cure can occur in cases with a normal cerebrospinal fluid, there exists very little evidence to suggest the possibility of its occurrence in cases where there have at any time been gross abnormalities in this fluid.

This study of proved cases receiving antypol or pentamidine in prophylactic dosage established the fact that these drugs caused trypanosomes to remain scanty or absent in the peripheral circulation for at least 7 months. This fact has two consequences of practical importance in the control of the epidemic. First, missed cases receiving prophylaxis are for the time being rendered comparatively harmless to the community by reason of their greatly reduced capacity for infecting tsetse. Secondly subsequent diagnosis of such cases is rendered much more difficult. The ultimate behaviour of the trypanosomes in these cases is unknown, but for practical purposes it is probably wise to postulate that they may return to the circulation in greater numbers after a further period has elapsed. If this proved to be so such missed cases would constitute a serious handicap in the eventual control of the epidemic. The degree of this danger can only be estimated by more prolonged observation, which is now proceeding. Meanwhile, as a safeguard, a further mass diagnosis of the area is to be undertaken 1 year after the prophylactic experiment started, and this will be followed by a second campaign of mass prophylaxis in which each person will receive a single injection of pentamidine only.

#### B. RESULTS OF MASS PROPHYLAXIS IN THE FUERO AREA

This section is concerned with the population in the Fiero area in whom no infection could be found immediately prior to prophylaxis. Table XI summarizes the results of a follow up at 7 months.

The cases shown under the heading '1 abnormal C.S.F. (gland and blood negative)' were obtained in the following way. At the re-examination all the subjects were scrutinized carefully and asked if they had any symptoms suggesting sleeping sickness and if the slightest suspicion arose from the appearance or history the subject was lumbar punctured and the cells counted. Count of over 5 per c.mm. (In borderline cases 2 or 3 c.mm. were scrutinized) was taken as *prima facie* evidence of infection. In the area under consideration there is no other non-acute disease likely to cause an increase of much above 5 cells except in very rare instances, and the cases shown may be accepted as true cases of sleeping sickness.

This table reveals a striking contrast between the number of infections diagnosed in the prophylactic groups and in the controls. Peripheral trypanosomes were revealed in only 0.1 per cent. in the former as against 5.1 per cent. in the latter while if cases presumptively diagnosed by lumbar puncture are added the respective figures are 1.0 and 5.7 per cent. The contrast is the more striking when it is remembered that some of the cases may have been already infected though undiagnosed at the time of prophylaxis. It remains

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TABLE XI  
RESULTS OF FOLLOW-UP 7 MONTHS AFTER PROPHYLAXIS

Drug	Dosage	Number in group	Number re-examined at 7/12	Gland +	Blood +	Gland or blood + %	Abnormal C S F (gland and blood negative) %	Total S S %
Antrypol	1 gramme	483	437	0	2	0.5	4.09	1.4
Pentamidine isethionate	Mg 150-200	692	549	0	0	0.0	4.07	0.7
Pentamidine isethionate	375-400	590	512	0	0	0.0	5.10	1.0
Total prophylaxis		1,765	1,498	0	2	0.1	13.09	1.0
Controls—no prophylaxis		518	471	12	12	5.1	3.06	5.7

to attempt an estimate of the proportion of cases found which may be thus accounted for, it is, fortunately, possible to arrive at an approximate figure from a calculation based on observations over 7 months, described in previous sections on untreated cases and on known cases treated only with prophylactic doses of antrypol and pentamidine.

To consider first the cases diagnosed by gland puncture or blood film. It has already been shown that at least 35 per cent of existing cases are missed on one examination by these procedures. Now in the original diagnosis of the area preceding prophylaxis an overall incidence of 4.8 per cent was obtained, from which it may be calculated that about 2.6 per cent of the population remained as undiagnosed cases, this figure applies to both the prophylactic and the control groups since care was taken to ensure that the infection rate was comparable in both. In the control group, since again only about 65 per cent of these missed cases would be diagnosed at the final examination at 7 months, it follows that such cases account for an incidence of about 1.7 per cent out of the incidence of 5.1 per cent actually found, leaving a figure of 3.4 per cent as representing the incidence of new cases in this group diagnosable by gland puncture and blood film. In regard to the combined prophylactic groups on the other hand, it can be seen by reference to Table IX that only some 11 per cent of missed cases may be expected to reveal trypanosomes at 7 months, so that such cases could account for an incidence of 0.3 per cent in these groups. Since only two cases were actually found positive at 7 months, giving an incidence of 0.1 per cent, it is probable that these two cases had already been infected before prophylaxis.

To consider next the cases presumptively diagnosed by a raised C S F cell count 7 months after prophylaxis. Here again observations carried out on known infected cases after the administration of antrypol and pentamidine in prophylactic dosage can be used as a basis from which to calculate approximately the proportion attributable to previously missed cases. It will be remembered that the cases shown in column 8 of Table XI were selected for lumbar puncture because they admitted to symptoms suggestive of sleeping sickness—in fact they combined the features of suggestive symptoms and a raised C S F

cell count with absence of peripheral trypanosomes. Now in study of 84 known cases of sleeping sickness who received prophylactic doses of antypol or pentamidine it had been found that 7 or 7.4 per cent. fell into this category on re-examination after 7 months. Applying this figure to the general prophylactically treated population and remembering that about 2.6 per cent. of this population may be assumed to have been suffering from undiagnosed infection at the time of prophylaxis, we arrive at a figure of 0.2 per cent. as representing previously missed cases which may be expected to reveal themselves by lumbar puncture at 7 months; in other words about three cases. In actual fact thirteen cases were found (Table XI) which suggests that some new infections had occurred after prophylaxis but had remained cryptic.

The actual C.S.F. cell counts obtained in these thirteen cases have some bearing on the problem. They were as follows: six to twenty cells, two cases; twenty-one to 100 cells, three cases; over 100 cells, eight cases. The fact that all except two cases had a count exceeding twenty cells, and that in the majority it exceeded 100 per c.mm. is a point in favour of a long standing infection contracted prior to prophylaxis, but in the absence of any concrete evidence to show how early the C.N.S. may become involved in the type of case met with in this area, too much stress should not be laid on this point. After reviewing the evidence, we feel that the occurrence of cryptic new infections by the Fuero strain after prophylaxis should be regarded as a definite, though unproved, possibility. It is hoped that a later follow up in the area will shed more light on this point.

#### C. RESULTS OF MASS PROPHYLAXIS IN TWO NORMAL AREAS.

For comparison with the results of prophylaxis in the Fuero area, two other areas were selected for a similar trial. One, a political area known as Mofindo section, is situated in the neighbourhood of Kailahun some 30 miles south of Fuero; the other known as Toli Section, lies in the extreme eastern tip of Sierra Leone about the same distance south-east of Fuero. In both these areas the disease is of the classical type marked by almost constant enlargement of the cervical glands in which trypanosomes can usually be found with ease.

In both areas prophylaxis was preceded by a routine diagnostic survey and treatment of the cases found. Then, in Mofindo Section, the population was divided by random sampling into three roughly equal groups of which one received pentamidine isethionate, the second antypol and the third received no drug but served as a control. In Toli Section the population was divided into two groups only: one of which received antypol and the other pentamidine, since in this case two adjoining sections situated one on either side of Toli were used as controls. These two sections, known as Kundu and Ndakalede, had been diagnosed and treated in the same way as Toli just prior to prophylaxis of the latter and were then left undisturbed until the end of the experiment.

The results are shown in Table XII. It was unfortunate for this experiment that the incidence of sleeping sickness just prior to prophylaxis was comparatively low, so that even without prophylaxis not a great number of new

TABLE XII  
TWO SLEEPING SICKNESS AREAS OF NORMAL TYPE. INFECTIONS DISCOVERED AFTER 10 MONTHS IN  
POPULATION WHO HAD RECEIVED PROPHYLACTIC ANTRYPOL OR PENTAMIDINE  
AND IN UNTREATED CONTROLS

Area	1945 SS incidence %	Drug	Dosage	Population under test	1946 Cases after 10/12	1946 Incidence %
I Mofindo	4.0	Antrypol	1 x 1 gramme	309	—	0.19
		"	2 x 1	213	1	0.00
		Pentamidine isethionate	1 x 100 mg	153	—	0.00
			1 x 150 "	239	—	
			350 mg in 2 inj	160	—	
		Nil	—	548	2	0.36
II	Toli	Antrypol	1 x 1 gramme	80	—	0.40
			2 x 1	170	1	0.16
		Pentamidine isethionate	1 x 150 mg	237	—	0.00
			2 x 150	132	—	
	Kundu	Nil	—	846	6	0.71
	Ndaka/c/c	Nil	—	1,013	11	1.38

cases were to be expected in 10 months, but outside the Fuero area it is no longer possible to find a region in Sierra Leone with any but a low incidence. In the event, in Area I, one case was found among 1,074 people who had received prophylaxis, while two cases were discovered in 548 controls. In Area II the figures were one among 619 prophylactically treated people and twenty among 1,859 controls.

In Area II it is quite clear that prophylaxis has been very effective. In Area I, since only two controls became infected, the chances of exposure to infection of the prophylactically treated people were obviously quite low. Nevertheless, this in itself may well be a significant fact. Normally, it is very rare in this region for one mass diagnosis and treatment so to reduce the prevalence of infection that, 10 months later, it has dropped to less than one-tenth of its former figure as had occurred amongst the controls in this case (from 4.0 per cent to 0.36 per cent), the more normal reduction is to anything from about one-half to one-fifth (cf. Area II). The large reduction in this case is probably explicable on the grounds that two-thirds of the population of Area I

were not only rendered immune to infection themselves but were also incapable of becoming a source of infection to others so that the chances of infection to which the controls were exposed was thereby much reduced. This factor may be of considerable general practical importance in large scale prophylaxis.

The fact that no infections were found among people who had received pentamidine whereas two were found among those who had received antrypol is suggestive, but in view of the paucity of new infections inconclusive.

It should be added that number of people in the prophylactically treated groups who admitted to headache were lumber punctured but in no case was a raised C.S.F. cell count obtained, and in fact in all cases this symptom was judged on clinical grounds to be due to causes such as chronic malaria or anaemia and not to sleeping sickness. There was therefore no suggestion that cryptic cases might have occurred.

## VI. DISCUSSION AND CONCLUSIONS

In the two normal areas mass prophylaxis was most effective. Taking both areas together the results after 10 months were as follows. After 1 to 2 grammes antrypol two cases were found in 772 people examined (0.26 per cent.) after 100 to 300 mg. pentamidine isethionate, no cases were found in 921 people examined. In the untreated controls, twenty two cases occurred in 2,407 people (0.92 per cent.). The prophylactically treated population was subjected to a slightly smaller risk of exposure to infection than the controls since a greater proportion lived in Area I but this factor is not large enough seriously to affect the issue (had the same number of controls as of prophylactically treated people been taken for observation in each area the expected infection rate among them would still have been five times as high as among the antrypol and pentamidine groups combined, viz. 0.62 per cent. as against 0.12 per cent.). From these results it appears to us that mass prophylaxis may have a very important role in certain circumstances, particularly in the case of an epidemic of normal type with a very high incidence in the population. For instance, in 1940 there was in Sierra Leone an area of infection of normal type with an incidence exceeding 20 per cent. With the employment of repeated mass treatment and the provision of a dispensary it took some 3 years to reduce the incidence to below 1 per cent. had it been possible to give all healthy people prophylaxis at the time of the first diagnosis and treatment of the infected, there seems little doubt the incidence would have been reduced to this figure at once. It must be remembered that if no untreated controls are allowed to remain in the area, as has been necessary for our observations, the possibility of re-infection after the prophylactic effect of the drug has worn off is greatly reduced.

In the Fucro area mass prophylaxis may not eventually prove to have been so effective owing to the persistence of mixed cases, but the net results are likely to be even more valuable than in a normal area since the situation was out of hand and no other methods of control offered much hope of success.

It remains to discuss the respective merits of antrypol and pentamidine in mass prophylaxis. First, as to efficacy in preventing new infections accompanied by the presence of peripheral trypanosomes. In all areas (i.e. the Fucro

and the normal) combined, none of 1,892 people who received pentamidine revealed trypanosomes 6 to 10 months later, of 1,209 people who received antrypol, four revealed trypanosomes, but of these two probably represented pre-existing infections. It is difficult, therefore, to be sure that pentamidine was more effective, though the result is suggestive.

Secondly, as regards ease of administration and freedom from side effects. It is a considerable advantage in dealing with large numbers that pentamidine isethionate can be given intramuscularly, and is relatively painless. The frequency of the commoner reactions experienced in the district covered by the map is shown in Table XIII.

TABLE XIII  
IMMEDIATE REACTIONS FOLLOWING INJECTION OF PROPHYLACTIC DRUGS

Drug	Cases injected	Reactions per cent					
		Vomiting	Mild syncope	Colic	Pyrexia	Urticaria	Total
Antrypol	3 572	0 45	0 14	0 08	0 08	0 20	0 95
Pentamidine isethionate	13,509	0 18	0 13	0 24	0 04	0 01	0 59

It will be seen that immediate reactions were commoner after antrypol. None of the reactions was serious though vomiting after antrypol and abdominal colic after pentamidine were troublesome. The incidence of vomiting after antrypol was much reduced by giving the injection really slowly. The colic sometimes experienced after pentamidine usually commenced about  $\frac{1}{2}$  hour after injection and persisted for about 2 hours, it appeared sometimes to be a genuine colic, sometimes a persistent pain. No deaths occurred after antrypol. One patient died 4 days after receiving pentamidine and was stated to have been unwell since the injection, but as her illness was not reported until after death, she was not seen. In view of the large numbers involved and the frequency of death from unknown causes in Africa, it is not possible to say whether death was or was not attributable to the drug.

A late effect of antrypol should be mentioned. It was quite frequent for patients to complain of pains in the soles of the feet coming on a day or two after injection and persisting often for a week or more. In a few of the worst cases, some peeling of the soles was noticeable and ordinary walking became difficult.

The highest adult dose of pentamidine found to be generally well tolerated was 175 mg, and when 200 mg doses were given the incidence of immediate reactions rose steeply to nearly 1 per cent.

We conclude that in Sierra Leone pentamidine isethionate has shown itself at least as efficient in prophylaxis as antrypol, and that in addition it presents the advantages of fewer side effects and greater ease and rapidity of



## METHODS.

ESTABLISHMENT OF *B. duttoni* ON EGGS.

The culture on eggs was successfully established by the inoculation of blood from mouse which had previously been infected with blood from human case. Under conditions of strict asepsis, working in a sterile cabinet, 0.4 c.c. of heart blood from the mouse was immediately inoculated into eight fertile eggs on the 7th day of incubation using a tuberculin syringe fitted with 26 G 14-inch long needle; the inoculation was made through hole punched in the blunt air sac end into the middle of the egg. This hole was then sealed with paraffin-sealing-wax mixture. The eggs were then incubated further 7 days at 37° C. They were then opened. The blood vessels of the chorio-allantoic membrane were torn across and allowed to bleed into the allantoic fluid. Examination of moist preparations by dark ground illumination and of stained smears showed the presence of numerous spirochaetes. The mixture of blood and allantoic fluid served as an inoculum for further batches of eggs, each egg receiving 0.1 c.c. The strain has thus been subcultured weekly for ten passages. The eggs show variable growth of spirochaetes, the growth in some being very profuse in others very scanty.

## PREPARATION OF ANTIGEN FOR COMPLEMENT FIXATION TEST

Eggs were inoculated as described above. After incubation for 6 to 8 days, the eggs were transilluminated. Dead eggs were discarded. Those with living embryos were opened at the blunt air sac end with a flame. The shell membrane was reflected. With another pair of sterile forceps, the chorioallantoic membrane was torn, and the blood vessels allowed to bleed into allantoic fluid. A sample of this fluid was then examined by dark ground illumination to determine the degree of infection. If this was profuse, showing 50 to 100 spirochaetes per high power field, the fluid was pipetted into a sterile centrifuge tube. During these manipulations care was taken not to rupture the yolk sac to avoid an admixture of yolk granules.

The harvested fluid was then centrifuged at low speed, 1 000 revolutions per minute for 5 minutes, to throw down the red blood cells. The supernatant fluid was then pipetted into a further set of sterile tubes and centrifuged at 2,000 revolutions per minute for 3 hours. At the end of this time the spirochaetes had been thrown down to form a thin white sediment at the bottom of the tube. The supernatant allantoic fluid was then removed and replaced with sterile physiological saline containing 0.3 per cent. phenol. The tube was then shaken and an even suspension of spirochaetes was obtained. This suspension constituted the antigen for the test. Its anti-complementary properties were found to be low and the suspension could be used undiluted for the test.

## PROCEDURE FOR THE TEST

1. The sera under test were heated at 56° C. in a water bath for 30 minutes.
2. Serial dilutions of the sera were made in normal saline as follows  
1 12.5, 1 25, 1 50 1 100 up to 1 3,200
3. 0.1 c.c. of each dilution was pipetted into a 3 inch ×  $\frac{1}{2}$  inch tube.
4. An extra tube was set up as the anti-complementary control of the serum.



The results obtained from 24 sera sent in for routine blood grouping tests were all negative.

Thirty sera giving a strongly positive Wassermann reaction were tested, one was anti-complementary four gave a  $\pm$  result in a dilution of 1:125, and the remaining twenty five were negative.

Table II gives the results obtained from seven sera from cases suffering from various disease conditions. One from a case of typhus fever showed fixation in a dilution of 1:125. The remainder were negative.

TABLE II.

Case number	Serum from case of	Serum dilutions					Anti-complementary control
		1:125	1:25	1:50	1:100	1:200	
1	Typhus fever	$\pm$	—	—	—	—	—
2	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—
4	Rheumatic fever	—	—	—	—	—	—
5	Malaria chronic	—	—	—	—	—	—
6	Virus pneumonia	—	—	—	—	—	—
7	Tuberculosis	—	—	—	—	—	—
8	Relapsing fever + control	+	+	+	+	$\pm$	—

From these results, it is apparent that in each of the six known cases of relapsing fever in which the diagnosis was established by finding the spirochaetes in blood smears, the test gave a positive result. The lowest titre of complement fixation was in Case 2, in which it was given in a serum dilution of 1:25. It is tentatively concluded that fixation of complement in a titre of 1:25 and over should be taken as diagnostic of relapsing fever.

The time of development of these antibodies has not been determined with accuracy as the duration of illness in most of the cases is unknown. Blood was taken from one case at the end of what was probably his first attack of fever and then again a week later. The results of the complement fixation tests on the sera from these bloods are given in Table III.

In this one case it appears that complement fixing antibodies were present in low though provisionally diagnostic titre at the end of the first bout of fever. A week later the titre had risen to well above the diagnostic level.

How long the antibodies persist after apparent cure has also not been established. This question will be investigated as the opportunity occurs.

Blood film examination for spirochaetes was negative in four of the six known cases of relapsing fever at the time the blood for the complement fixation test was taken.

TABLE III

	Serum dilutions						Anti-complementary control
	1 12 5	1 25	1 50	1 100	1 200	1 400	
First specimen, interval one week	+	±	+	—	—	—	—
Second specimen	+	+	+	+	±	—	—

## SUMMARY AND CONCLUSIONS

A complement fixation test for the diagnosis of relapsing fever, using an antigen prepared from egg cultures of *Borrelia duttoni*, is described

This test promises to be of value in those cases of relapsing fever, in which finding the causative spirochaete by direct examination of blood films is difficult or impossible. It should also be useful in carrying out surveys to determine the incidence of relapsing fever in a population. It may prove of value in cases of relapsing fever, as has the Wassermann test in cases of syphilis, in deciding when a patient has been cured of his infection, though this aspect of the test has not yet been studied.

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# THE EFFECT OF DIET AND HELMINTHIC TREATMENT ON AFRICAN SCHOOL CHILDREN

BY

O D MACNAMARA, M B, B CH \*

The large majority of Africans in Nigeria suffer to a greater or lesser degree from undernutrition and an ill-balanced diet. They are also mostly infected with helminths.

The undernutrition is largely due to the fertility-economic position, any increase in wealth resulting rather in an increase in numbers of the population than in an increase in the standard of living. The helminthic infection results from the crude ideas of sanitation possessed by the people.

The people who are reviewed below live in central Nigeria, where the food products of the forest zone and savannah country can both be easily obtained. An investigation was undertaken to find to what extent school children would be benefited by a proper diet and by treatment for helminthic infection.

The staple food of the people consists of a kind of thick porridge made by grinding and cooking guinea corn. Palm oil or groundnut oil is usually added after cooking. A soup made up with green leaves and peppers is also taken, but the leaves themselves are not eaten. Dried fish is usually taken in small amounts once a day, and occasionally groundnuts, meat, yams, cassava, millet and soya beans are also eaten. Rice is becoming increasingly popular and tends to replace the guinea corn. Mango is the only fruit commonly eaten. Sour milk is taken in small amounts, but is not popular, nor are eggs, which are believed to cause sterility.

It will thus be seen that the diet is ill balanced, being largely carbohydrate, while protein is deficient. The main source of protein available is the vegetable protein in groundnuts, and this is low in methionine and lysine (BLOCK and BALLING, 1944). Deficiency in protein and especially in methionine, may be the cause of cirrhosis of the liver, which is found not uncommonly in adults and children.

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were fully examined, their weights, heights, unexpanded chest measurement and endurance being measured. Endurance was estimated by the time for which the child could hang by his hands from a horizontal bar

Table IV shows the results obtained.

TABLE IV  
RESULTS OF INVESTIGATION

Group.	Weight.			Height.			Chest.		
	Average initial weight. st. lb. oz.	Average gain. lb. oz.	Standard deviation of gain. lb. oz.	Average initial height. ft. in.	Average gain. in.	Standard Deviation of gain. in.	Average initial measurement. in.	Average gain. in.	Standard deviation of gain. in.
A. Control	4 4 4	1 10 2	3 0	4 5 7	0 80	1 11	24 1	0 059	0 23
B. Fed	4 3 10	3 10 4	4 4 6	4 4 0	1 08	0 632	22 2	1 10	0 72
C. Deparasitized	4 7 3	0 8	7 8	4 7 7	0 5	0 61	23 3	0 27	0 705
Twice standard error of group			1 14 5			0 51			26

Group.	Endurance Time.			Vitamin deficiency	
	Initial time. min. sec.	Average gain. sec.	Standard deviation of gain. sec.	Number of cases initially	Number of cases at end.
A	18	18 5	37 0	1	2
B	1 45	18 8	43 6	6	0
C	2 35	15 8	37 0	3	3
D				8	3
Twice standard error			18 5		

The weights, heights, etc., of Group D have not been given as they are not comparable with the other groups.

**Weight.**—The children in Group B (expensive diet) gained an average of 3 lb 10½ oz., whereas the control Group A gained an average of 1 lb. 10½ oz. and those who had had treatment for parasites, Group C, gained an average 9½ oz. Thus those children on the large extra diet gained on an average a gain

ificantly more weight than the control group or the children who had anti-helminthic treatment. The children who had had anti-helminthic treatment gained less than the control, but the difference was not significant.

*Height*—There was no significant improvement in gains in height in either Groups B or C compared with the control group.

*Chest*—There was a significantly greater gain in Group B, but no significant difference in the other two groups.

*Endurance Test*—Here there was apparently a significant gain in Group B compared with the other two.

### *Vitamin Deficiency*

After the extra feeding in Group B, no children showed any signs of deficiency diseases although six of the fifty-four had shown signs of vitamin B<sub>2</sub> deficiency before the extra ration began. In the deparasitized children, Group C, there was no change in the incidence, and in the control group there was an increase by one. In Group D there was a drop from six cases to three, all being cases of vitamin B<sub>2</sub> deficiency.

### DISCUSSION

From these results it appears that routine treatment for helminths had no marked beneficial effect on the children. It was found that of those treated for hookworm, 65 per cent became re-infected in 3 months. It is also a possibility that repeated courses of chenopodium or tartar emetic, which would be needed to keep a child free from helminths, might be more deleterious to the health than the parasites themselves.

The children, Group B, given the extra diet showed no significant extra gain in height, although the gain was slightly more than in the other groups, and it appears that the extra diet had no marked effect on the rate of skeletal growth. The significant extra gain in chest measurement in the Group B, compared with Groups A and C, may partly indicate an increased improvement in the form of skeletal growth with reduction of flat chests. The increase of chest measurement is rather a measurement of flat chests. The increase of good posture, general well-being and lack of pulmonary disease, than a measure of rib growth. Chest measurement would also be increased by the deposition of subcutaneous fat.

The figures for endurance test are curious. Groups A and C had a high initial endurance which became less, but Group B had a lower initial endurance which increased. As such factors as discipline and morale would affect endurance tests and as Group B had a different set of teachers from Groups A and C, the figures for the groups are not really comparable. If the figures are accepted, a slight drop is found in Groups A and C compared with a significant increase in Group B.

It has been found that children with diets deficient in vitamins A and C have less endurance than children who are not so deficient (Kohn *et al*, 1943).



but feeding children on vitamin pills has not been found to improve the endurance (YUDKIN 1944). Among those children fed on an increased general diet (Group B), it appears there may be a resulting increase in endurance.

The full extra diet of Group B completely eliminated vitamin deficiency but the cheap extra diet of Group D although apparently supplying the needed vitamins, failed to eliminate signs of deficiency of riboflavin and nicotinic acid.

The general health of the children who had received the full extra diet (Group B) seemed better than the others. Helminthic treatment appeared to have no effect on the general health and the cheap diet only a slight effect. Owing to the groups being in separate schools, it was not possible to compare the effects of diet on mental ability but the teachers of the extra fed and treated children (Group C) said they had not noticed any improvement. Although children with high intelligence have usually been found to be better nourished than children with low intelligence (ENGLAND, 1936; MACKENZIE, 1944), it may well be that such children are the offspring of intelligent parents who take more care of diet and the extra intelligence of the children may be due more to heredity than nutrition. In improving the nutrition of the school child in Africa, the main object is to produce a better man capable of more work and of being a better citizen, but doubt has been expressed if improving nutrition will ever improve intelligence, scholastic performance or morals (CAHILL, 1945).

### SUMMARY

The experiment conducted in central Nigeria has shown that the African child here is undernourished, to a certain extent lacking in vitamins, and that he is richly infested by parasites. By giving the child a good diet there is a marked gain in weight, disappearance of vitamin deficiency, an increase in chest measurement and well-being, also possibly a marked increase in physical endurance.

Routine helminthic treatment of school children appears to be almost useless in a highly infected community. The extra supply of a cheap diet of the usual native foods such as groundnuts is insufficient to prevent deficiency diseases.

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# FOLIC ACID IN SEVERE NUTRITIONAL ANAEMIA A REPORT OF FIVE CASES

BY

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Severe anaemia is responsible in Malaya for much ill-health, and the death-rate is high. The causes are various—malaria, intestinal worm infestation, malnutrition and chronic infections. During the past 9 months, 142 cases of anaemia have been investigated in detail—a small proportion of the total of such cases which have passed through the 400-bed hospital adjoining this Institute in the same period. Of these 142 cases, twenty-four were found to have a red cell count of less than one million per c mm, and analysis of these twenty-four shows that nutritional macrocytic anaemia accounted for nine (three being associated with pregnancy), dimorphic anaemia (TROWELL, 1942, 1943) for fourteen, and iron-deficiency anaemia for one only. A nutritional factor apart from iron was thus concerned in twenty-three cases.

Five of these twenty-three cases were treated with oral synthetic folic acid, a small quantity of which had been placed at the disposal of the Institute in February of this year. This paper records the response to treatment. All five were adult Indians, four female and one male. Before treatment all had a megaloblastic marrow, hyperbilirubinaemia, but no central nervous system involvement. Practical difficulties prevented gastric analysis in more than one case.

Cases 75 and 90 were of nutritional macrocytic anaemia, the former being pregnant. Cases 95, 96 and 100 were of dimorphic anaemia. In our library I can find no account of the use of folic acid in the treatment of dimorphic anaemia.

## METHODS

Venous blood was obtained without stasis, generally from an arm vein, but on two occasions in Case 95 it had to be taken from the external jugular, 3 c c were added to a bottle containing 3 mg of Heller and Paul's dry oxalate mixture (HELLER and PAUL, 1934).

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The haemoglobinometer used was of the Sahli type, standardized so that 100 per cent. represents 14.0 grammes of haemoglobin per 100 c.c. of blood. The figures given in Tables I to V are the original results translated to the 14.5 standard.

Packed cell volumes were estimated by centrifuging oxalated blood in Wintrobe tubes for 30 minutes at 3 000 revolutions per minute.

The copper sulphate specific gravity method was used in estimating plasma proteins. (PHILLIPS *et al.*, 1945)

CASE 75 Female Indian, aged 25 (Table I and Fig. 1)

This young rubber estate labourer was admitted on 21.2.47 complaining of abdominal pain with diarrhoea for 5 days and increasing exhaustion for the past 6 months. She was 6 months pregnant.

On examination, he was noted to be well-built and reasonably well-nourished woman. There was no dehydration, and the temperature was normal. There were no signs of rytic disease or of deficiency disease apart from intense anaemia. The tongue was normal and there was no jaundice, glandular enlargement, oedema or koilonychia. The spleen and liver were not palpable. Urine was normal. No malaria parasites were found in the blood.

The stools, about three per day were watery and contained mucus but no blood. They returned to normal in 2 days after starting sulphaguanidine therapy. No ova or amoebae were detected. The temperature remained normal throughout.

*Blood Findings*

The initial blood picture was of macrocytic orthochromic anaemia. The results of this and subsequent examinations are given in Table I. Fig. 1 shows diagrammatically the response to treatment. Sternal marrow examined on 25.2.47 was found to contain small but significant proportion of megaloblasts.

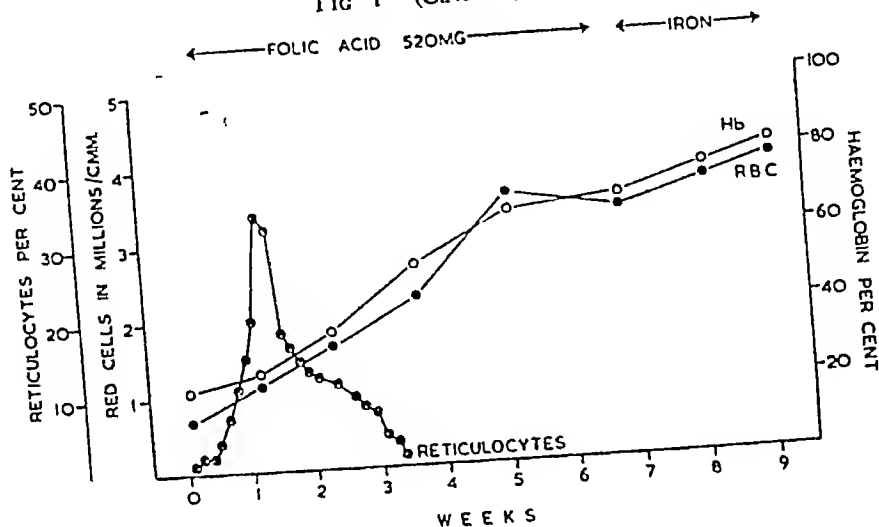
TABLE I  
CASE 75.

Date.	Day num- ber	Hb. % at 14.5	Hb. g.	RBC. milli- per cmm	Cal vol	PCV	MCV c.p.	MCHC %	Retac. peak %	Plasma protein g. per 100 ml.	Plasma bilirubin mg per 100 ml.
24.2.47	1	30.8	3.91	0.83	1.65	8.0	123.0	37.6		5.78	—
3.3.47	8	—	—	—	—	—	—	—	34.0	—	—
4.3.47	9	25.1	3.64	1.18	1.09	11.8	109.0	31.8		5.93	—
12.3.47	14	33.7	5.18	1.06	1.03	18.8	112.8	27.8		—	—
21.2.47	23	53.0	7.70	2.28	1.18	23.8	113.8	29.7		5.23	—
31.3.47	33	65.6	9.52	3.48	0.91	30.8	87.8	81.4		5.78	—
12.4.47	48	66.5	8.66	3.34	1.00	23.8	97.8	79.7		5.15	—
21.4.47	54	73.4	10.60	3.85	0.95	33.0	82.8	81.8		9.18	—
28.4.47	61	80.1	11.02	5.93	1.02	34.8	85.8	32.8		5.97	—

— Represents not estimated      O Represents negative.

On 26 2 47, folic acid therapy was begun. It was given as follows: 20 mg orally daily for 15 days, followed by 10 mg orally daily for 14 days, and finally 20 mg orally twice weekly for four doses. Total folic acid given was 520 mg in thirty-three doses.

FIG 1 (Case 75)



On the evening of the 1st day of folic acid therapy she went into labour, and delivered a 6 months' foetus. There was no post-partum haemorrhage and convalescence was normal. There was an immediate and sustained improvement in the blood picture, the reticulocytes reaching a peak of 34 per cent on the 7th day of treatment. Symptomatic improvement was also rapid. On 28 4 47, she was discharged at her own request, feeling fit.

#### CASE 90 Female Indian, aged 50 (Table II and Fig 2)

She was admitted on 17 3 47, complaining of extreme weakness and a sore tongue for 2 months.

She was a frail, poorly nourished little woman. Mucous membranes were intensely pale and the tongue pale and rather smooth, but not bald. The skin of the legs bore a mosaic pattern, but there were no other signs of "deficiency disease". There was a haemic murmur at the mitral area but no organic disease was detected in the cardiovascular or any other system. There was no jaundice, glandular enlargement, koilonychia or oedema. The liver and spleen were not palpable and no malaria parasites were found in the blood. Urine was normal and so was the temperature throughout.

On the 19 3 47, she was found to have a macrocytic orthochromic anaemia, and the following day sternal puncture revealed a fairly active megaloblastic marrow. Table II shows the initial and subsequent blood findings, and Fig 2 the response to treatment.

On 20 3 47, folic acid therapy was begun and given as follows: 10 mg orally daily for 14 days, followed by 10 mg orally twice weekly for two doses.

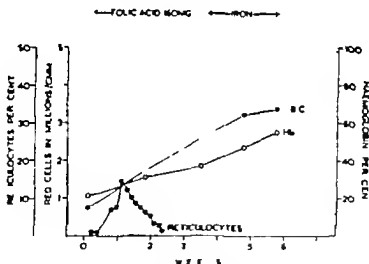
TABLE II.

AGE 90

Date.	Day number	Hb. at 14 d.	Hb. g.	R.B.C. mlla. per cmm.	Col. ind.	P.C.V. %	M.C.V. c.µ.	M.C.N.C. %	Retic. peak %	Plasma protein g. per 100 ml.	Plasma bilirubin mg. per 100 ml.
19.3.47	-1	20.2	2.94	6.72	1.12	8.5	116.6	34.8	14.0	6.25	—
26.3.47	6	—	—	—	—	—	—	—		—	—
31.3.47	11	31.6	4.62	1.69	0.84	17.6	103.6	25.6		6.05	—
12.4.47	22	32.6	4.90	2.50	0.65	23.4	90.2	20.6		7.22	—
21.4.47	32	46.2	6.72	3.24	0.7*	28	87.1	22.8		6.90	—
22.4.47	39	84.1	7.84	3.24	0.61	28.0	82.8	28.0		7.77	—
3.6.47	78	61.6	6.90	4.11	0.75	30.6	72.0	29.9		7.77	0

— Represents not estimated. 0 Represents negative

FIG. 2. (Case 90)



Total folic acid given was 160 mg. in 16 doses. Reticulocytes reached their peak of 14 per cent. on the 7th day of treatment.

She was discharged at her own request feeling fit on 28.4.47 after 41 days in hospital. Her blood picture then was normocytic and hypochromic.

Five weeks later on 3.6.47 she was re-admitted on account of acute bronchitis. She was found to have a frank iron-deficiency anaemia (she had no outdoor treatment) although her red cells had increased to over four millions.

CASE 95 Female Indian, aged 20 (Table III and Fig. 3)

She was admitted on the 22.3.47, complaining of pain in the left side and fullness of the abdomen for about a year.

Her history was that at intervals during the past 9 months she had been once in this and three times in another hospital. Previous records show that on each occasion her complaint was the same, and it was also noted each time that she was anemic and that her spleen was enormously enlarged. At no time had she any fever and many blood films had never revealed the presence of malarial parasites. She was, however, variously labelled as suffering from chronic malaria, "splenitis," and anaemia. She had been treated with Bland's pills and hepatox of the latter she received about 40 c.c. Following this therapy there was "considerable reduction" in the size of the spleen.

She came under my care on 31.3.47, having had hepatox 14 c.c. and ferrous sulphate 51 grains during the previous 9 days. For the next 9 days further treatment was withheld. She was found, on examination, to be a small, well-built and reasonably well-nourished individual. Mucous membranes were very pale, the tongue was pale and the papillae atrophic. There were no other signs of "deficiency disease," no oedema, glandular enlargement, jaundice, koilonychia or ascites. Temperature was normal and has remained so throughout. The liver was not palpable but the spleen was grossly enlarged, the tip being 22 cm. below the left costal margin. It was firm and appeared tender. There were no signs of other organic disease. The stool contained *Astarris* ova for which oil of chenopodium was given. Blood Kahn test was negative.

Gastric analysis on 2.4.47 showed a high fasting free HCl and total acidity, but after a meal the free HCl for the next 2 hours fell within normal limits at the upper level of normality; similarly she had a high normal total acidity.

Examination of the blood on 9.4.47 showed her to have a dimorphic anaemia. Results of this and subsequent examinations are shown in Table III. Fig. 3 shows diagrammatically the response to treatment.

TABLE III  
CASE 95

Date	Days since admission	Hb g (100 ml)	Hb g (50 ml)	RBC milli- per cmm	Col- ind	PCV	MCH	MCHC	Retic- peak	Plasma protein g per 100 ml	Plasma bilirubin mg per 100 ml
21.3.47	9	7.0	4.48	0.02	1.18	14.5	15.0	30.8	—	7.1	1.2
21.3.47	1	7.2	4.5	0.04	1.15	11.2	17.8	31.3	—	6.3	2.7
28.3.47	8	7.8	4.9	1.04	1.07	1.0	19.0	31.7	—	—	—
5.4.47	15	9.0	5.12	2.09	1.71	2.8	12.0	30.7	—	8.7	1.8
10.4.47	20	10.1	5.04	3.02	1.21	3.1	10.0	31.7	—	7.7	1.4
28.4.47	38	10.8	5.4	2.7	1.7	28.0	10.0	31.0	—	7.7	0.81
7.5.47	46	13.41	6.7	1.7	1.7	2.2	10.7	32.2	—	—	1.7
14.5.47	53	13.7	6.85	3.80	1.18	2.8	11.1	37.2	—	—	—

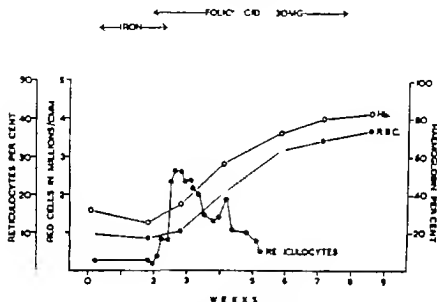
— Response to treatment

Serial plasma protein estimations on 10.4.47, 10.5.47, 14.5.47, 14.6.47, 14.7.47, 14.8.47, 14.9.47, 14.10.47, 14.11.47, 14.12.47, 14.1.48, 14.2.48, 14.3.48, 14.4.48, 14.5.48, 14.6.48, 14.7.48, 14.8.48, 14.9.48, 14.10.48, 14.11.48, 14.12.48, 14.1.49, 14.2.49, 14.3.49, 14.4.49, 14.5.49, 14.6.49, 14.7.49, 14.8.49, 14.9.49, 14.10.49, 14.11.49, 14.12.49, 14.1.50, 14.2.50, 14.3.50, 14.4.50, 14.5.50, 14.6.50, 14.7.50, 14.8.50, 14.9.50, 14.10.50, 14.11.50, 14.12.50, 14.1.51, 14.2.51, 14.3.51, 14.4.51, 14.5.51, 14.6.51, 14.7.51, 14.8.51, 14.9.51, 14.10.51, 14.11.51, 14.12.51, 14.1.52, 14.2.52, 14.3.52, 14.4.52, 14.5.52, 14.6.52, 14.7.52, 14.8.52, 14.9.52, 14.10.52, 14.11.52, 14.12.52, 14.1.53, 14.2.53, 14.3.53, 14.4.53, 14.5.53, 14.6.53, 14.7.53, 14.8.53, 14.9.53, 14.10.53, 14.11.53, 14.12.53, 14.1.54, 14.2.54, 14.3.54, 14.4.54, 14.5.54, 14.6.54, 14.7.54, 14.8.54, 14.9.54, 14.10.54, 14.11.54, 14.12.54, 14.1.55, 14.2.55, 14.3.55, 14.4.55, 14.5.55, 14.6.55, 14.7.55, 14.8.55, 14.9.55, 14.10.55, 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Folic acid therapy was begun on 22.4.47. It was given thus 20 mg. orally one dose only followed by 10 mg orally daily for 41 days. Total dosage 430 mg in forty two doses.

The reticulocytes reached their peak of 26 per cent. on the 6th day of treatment, and there has been a well-sustained improvement in the haemoglobin and red cell count.

FIG. 3. (Case 83)



After a month there was no apparent change in the size and texture of the spleen, and she complained frequently of pain and discomfort due to pressure. On 23.5.47 spleen puncture was carried out. Examination of the stained material did not reveal either malaria parasites or pigment. On the 26th, 27th and 28th of May thick films of peripheral blood did not show malaria parasites. On 28.5.47 paludrine therapy was begun in an endeavour to reduce the size of the spleen, should it be malarious. She received 0.1 gramme three times a week and after 4½ weeks (on 17.47), having had 1.4 grammes, it was noted that the tip of the spleen was only 15 cm. below the costal margin.

CASE 96. Female Indian, aged 26. (Table IV and Fig. 4.)

This rubber estate labourer was admitted on 1.4.47 complaining of fever on and off for 6 months, and of general weakness.

She was an intensely anaemic and poorly nourished young woman. The tongue was pale and the papillae atrophic. There was mosaic pattern on her legs and oedema of the feet and ankles. There was no glandular enlargement, jaundice, koilonychia or

ascites Her hair was dry and falling The liver and spleen were not palpable She was dyspnoeic and had a tachycardia of 100 to 140 per minute Temperature was 102.4° F Haemic murmurs were present at all areas of the heart, but no organic disease could be detected in the cardiovascular or any other system Urine was normal, no malaria parasites were found in the blood and no ova in the stools For the next 3½ weeks she ran an intermittent temperature which reached as high as 103.2° F, but was most commonly in the region of 101 to 102° F maximum No cause was discovered for this fever Examination of the blood on 9.4.47 showed her to have a dimorphic anaemia Results are given in Table IV

Marrow biopsy was carried out on 11.4.47 there was active megaloblastic erythropoiesis

As she appeared in no immediate danger, iron therapy alone was begun on 12.4.47 She was given ferrous sulphate 6 grains three times a day, and this was continued until discharge Two days later her general condition had deteriorated considerably Blood transfusion was not available and treatment was begun with proteolysed liver She received the equivalent of 90 grammes of liver solids daily, but it had to be stopped after 5 days as a fungus was discovered in the batch being used After a further 2 days (21.4.47) it was found that the blood picture had deteriorated, and next day she was desperately ill Oedema had increased and was now present round the eyes

Folic acid therapy was begun on 22.4.47, and given as follows 20 mg orally daily for 14 days, followed by 10 mg orally daily for 28 days Total dosage, 560 mg in forty-two doses

The reticulocytes reached their peak of 58 per cent on the 6th day of therapy (see Fig 4) Symptomatic improvement was rapid, and oedema disappeared rapidly

She was discharged from hospital at her own request, feeling fit, on 7.6.47

TABLE IV

CASE 90

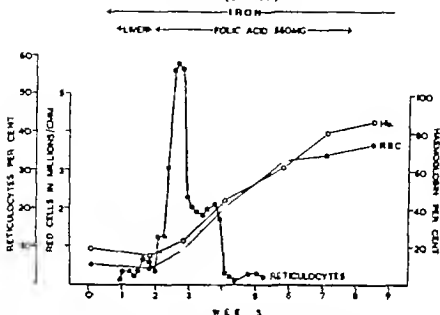
Date	Day number	Hb % at 14.5	Hb g	RBC mills per c.mm	Col ind	PCV %	MCV c μ	MCHC %	Retic. peak %	Plasma protein g per 100 ml	Plasma bilirubin mg per 100 ml
9.4.47	-13	18.3	2.66	0.53	1.74	8.7	165.7	30.5	58.0	7.25	0.82
21.4.47	-1	14.5	2.10	0.46	1.56	6.8	147.0	30.9		6.58	0.66
27.4.47	5	—	—	—	—	—	—	—		—	—
28.4.47	6	22.2	3.22	0.88	1.26	12.0	136.7	26.8		7.22	—
7.5.47	15	43.4	6.30	2.11	1.03	24.4	116.7	25.8		6.58	Very slight +
19.5.47	27	62.8	9.10	3.20	0.98	20.9	93.4	30.4		7.77	0
25.5.47	36	80.1	11.62	3.43	1.16	34.0	90.3	34.2		9.15	0
7.6.47	46	89.0	12.04	3.72	1.12	35.5	95.5	33.9		—	0

— Represents not estimated

O Represents negative



FIG. 4. (Case 96)



Case 100 Male Indian, aged 45 (Table V and Fig 5)

H. was admitted complaining of intense weakness and looseness of the bowels for 4 months.

On examination, he was found to be easily exhausted, and unable to raise his head from the pillow. He could not help feed himself and during the next few days was frequently delirious. He was under-nourished and very pale. The skin was dry and cracked, particularly on the legs, and his hair was dry and falling. He had intense halitosis. There were no mouth lesions, but the tongue was very pale and the papillae trophic. H. had no koilonychia, pseudic glandular enlargement, or oedema. Haemic murmurs were present at all areas of the heart although no organic disease was detected in the cardiovascular or any other system. Urine was normal. The spleen and liver were not palpable and there was no ascites. No ova were found in the stools. Temperature on admission was normal, but 4 hours later rose to 100° F and for the next 16 days was remittent, reaching 102° F on two occasions, and for the remainder of the time ranging between 99° F and 101° F. H. had no rigors during this time and malaria parasites were never found. Blood Kahn test was negative. No cause could be found for his fever. The temperature became normal on the 7th day of folic acid therapy. There was nothing suggestive of sprue in the appearance of the stools.

Examination of the blood on 10/4/47 revealed severe dimorphic anaemia. Results are shown in Table V. On the same date the normal marrow was found to be actively megaloblastic.

Folic acid therapy was begun and given thus 20 mg orally daily for 12 days, followed by 10 mg orally daily for 14 days. Total dosage 380 mg. in 26 doses.

The response to treatment was rapid (as shown in Fig 5), the reticulocytes reaching their peak of 55 per cent. on the 7th day. On the same day against

advice, he was walking about and asking to be discharged as he felt so much better

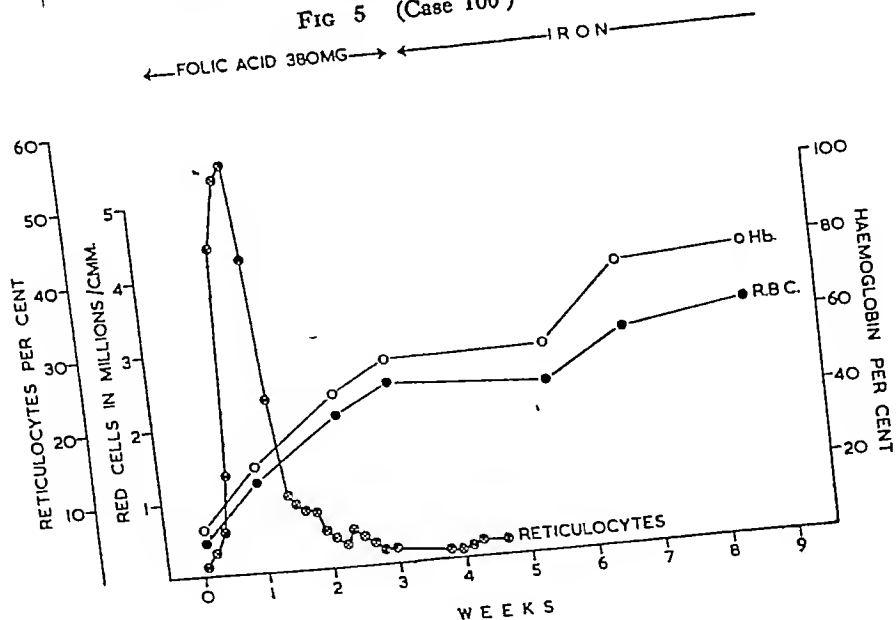
Folic acid was stopped and iron therapy begun on 3 5 47, as the MCHC was only 29.3 per cent This was continued until he was discharged, feeling well on 14 7 47

TABLE V  
CASE 100

Date	Day number	Hb % at 14 5	Hb g	R.B.C mills per c.mm	Col ind	P.C.V %	M.C.V c μ	M.C.H.C %	Retic. peak %	Plasma protein g per 100 ml	Plasma bilirubin mg per 100 ml
10 4 47	0	12.5	1.82	0.42	1.49	6.0	143.3	30.3	55.0	7.60	3.09
16 4 47	6	28.9	4.20	1.23	1.18	14.9	121.6	28.1		7.08	—
25 4 47	15	46.3	6.72	2.00	1.16	21.9	109.5	30.7		7.08	—
3 5 47	23	54.1	7.84	2.42	1.12	26.8	110.7	29.3		7.22	0.63
20 5 47	40	54.1	7.84	2.26	1.20	22.4	99.2	35.0		8.18	0.59
28 5 47	48	75.3	10.92	2.90	1.29	28.8	99.3	37.9		9.15	O
10 6 47	61	77.2	11.20	3.14	1.23	30.5	97.1	36.7		9.15	Very slight +
14 7 47	95	94.6	13.72	4.55	1.04	36.7	80.7	37.4		—	—

— Represents not estimated      O Represents negative

FIG 5 (Case 100)



## COMMENTS.

*Case 90*

Improvement was rapid and well sustained considering that no iron was given during her stay in hospital. Even after discharge, and without any further treatment, she continued to manufacture hypochromic microcytes.

*Case 85*

She had no symptoms or signs, apart from splenomegaly referable to hepatic cirrhosis, and no history of haemorrhages. The splenomegaly on a previous occasion had diminished on large doses of heparin—this time there was no apparent change after 4½ weeks of folic acid therapy. It is unfortunate from the point of view of experimental observation that paludrine was not withheld for some time longer. To what extent paludrine contributed to the reduction of the spleen must be conjecture—the experience of the Malaria Division of this Institute is that malarial spleens recede fairly rapidly on prophylactic paludrine.

Recovery was fairly rapid during the 6 weeks of folic acid therapy but after it stopped regeneration of red cells was slow and the haemoglobin fell slightly.

*Cases 98 and 100.*

Like Case 85 these were examples of dimorphic anaemia, but neither had a palpable spleen. They were both desperately ill before folic acid was given, and both had a pyrexia of unknown origin before treatment. In both the temperature became normal a few days after beginning treatment. Their response to treatment, both symptomatic and haematological, was dramatic and well sustained, the reticulocytes reaching 58 per cent. in Case 98 and 55 per cent. in Case 100 on the 6th and 7th day respectively.

It is difficult in this country to induce potential donors, even among close relatives, to give blood for transfusion.

The hatch of proteolysed liver used in treating Case 96 was impotent probably because, as already mentioned, a fungus was discovered growing in it, and it produced no reticulocytosis in two other cases of nutritional macrocytic anaemia. The reticulocytosis which did occur at the beginning of treatment of Case 96 was minimal (7 per cent.) and occurred on the 5th day of liver therapy. It was probably the primary reticulocytosis due to iron, typical of dimorphic anaemia.

Serial total plasma protein estimations were carried out on all five cases. In each case there was a marked increase between initial and final estimations. Each patient received an ordinary third-class hospital diet without the addition of any "extras."

In only one case (Case 96) was oedema present. The plasma proteins were 7.25 grammes per 100 ml. on admission, and fell to 6.58 grammes when oedema was most marked.

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Although serial bilirubin estimations were not carried out in Cases 75 and 90, the respective plasmas before treatment had a strong yellow tinge which gradually disappeared as the blood pictures improved

## CONCLUSIONS

Synthetic folic acid, administered orally, is clearly of value in the treatment of severe nutritional anaemia

Although the series of cases presented in evidence is small, this drug undoubtedly has life-saving potentialities when used in moribund cases of dimorphic anaemia—an attribute of prime importance in Malaya where nutritional anaemia is responsible for many deaths

The value of the drug in this country is further enhanced by virtue of the fact that red cell concentrates for transfusion are, for practical purposes, unobtainable

## SUMMARY

One hundred and forty-two cases of anaemia have been investigated in detail. Twenty-four had a red cell count of less than one million per c mm, and of these only one was due to pure iron deficiency, the remaining twenty-three were nutritional.

Five of these twenty-three cases were treated with oral synthetic folic acid. The response to treatment in all was satisfactory, and in two cases dramatic. These two were cases of dimorphic anaemia.

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 ——— (1943) *Ibid*, 37, 19





The fasting counts varied between 1 and 74 but only three were over twenty. When the fasting count was high, the count dropped before rising to peak after the meal. The time of the peak value was fairly constant and in all the curves lay between 2 and 3½ hours and was at 2½ hours in ten of the curves. The peak value was very variable averaging 180 (SD  $\pm$  43.5 range 100-270). The height of the peak varied by more than 50 per cent. from time to time in the same person. The error of the count on one specimen of serum was about  $\pm$  15 per cent. for a given observer and there is large subject element in estimating the count. Besides the actual error of counting there is great variation in the diameter of the particles, implying variation in particle volume of several hundredfold. Theoretically then, similar counts may represent very different amounts of chylomicron fat. Actually the relatively consistent findings suggest that, using standard meal of homogenized fat, the variation in particle size is similar in different persons, since comparable counts are obtained when the same amount of fat is used.

#### RELATION BETWEEN CHYLOMICRON RISE AND AMOUNT OF FAT ABSORBED

Changes in the curve with varying quantities of fat in the meal were studied in one subject. With the standard amount of 18 grammes, the peak values in three curves were 136, 162, 230 (average 176). With 9 grammes the peak was 80 and with 36 grammes the peak values in two curves were 410 and 330. The relationship between the height of the curve and the amount of fat given suggests that the height of the chylomicron rise is affected by the amount of fat available for absorption, but other evidence was obtained showing that the rise does not depend on the total amount of fat actually absorbed.

It was found that when a soluble calcium salt (e.g. 7½ grammes of calcium lactate) is given with the meal, a low curve results. The average peak in four people with such curves was only 83. To determine whether the low chylomicron count corresponded to a diminution in the amount of fat absorbed, a balance experiment was done on two normal subjects who showed low curves with calcium lactate. The diet contained 85 grammes of fat per day and 30 grammes of calcium lactate were given per day in divided doses with each meal for 6 days. The results are shown in Table I.

TABLE I.  
EFFECT OF CALCIUM SALTS ON A. ABSORPTION.

Subject.	Average daily total fat content of stool (gramme).		
	During 6 days before giving Ca lactate.	During 6 days of Ca lactate administration.	During 6 days after giving Ca lactate.
P.M.	9.3	11.8	11.4
P.R.	4.3	8	6.4
Subject.	Peak value of chylomicron count.		
	Without calcium lactate.	With calcium lactate.	
P.M.	130	43	
P.R.	120	80	

The low chylomicron count found when a calcium salt is given with the fat meal is not associated with any comparable diminution in total fat absorption

Further evidence on the relation between the chylomicron curve and the total amount of fat absorbed is derived from thirty-eight curves on sprue patients whose fat intake and output was being measured (Table II) The

TABLE II  
RELATION BETWEEN HEIGHT OF CHYLOMICRON COUNT AND PERCENTAGE OF INGESTED FAT  
ABSORBED

		Percentage dietary fat absorbed				
		<60	60—	70—	80—	Total
Chylomicron peak count	Number of curves <100	3	5	5	4	17
	Number of curves >100	3	4	6	8	21
	Total	6	9	11	12	38

chylomicron curves have been grouped according to the peak value, above and below 100 Table II shows that while an abnormally low chylomicron count is common when fat absorption is poor, the incidence of low counts is not quantitatively related to the severity of the fat absorption defect On three occasions normal curves were found when only 50 to 60 per cent of the ingested fat was being absorbed Such cases suggest that chylomicrons may represent only a fraction of the blood fat, which may rise during fat absorption though total fat absorption be poor, and conversely, as shown by the calcium lactate experiments, may fail to rise though total fat absorption be good

#### CHYLOMICRON COUNTS IN RELATION TO BLOOD FAT CURVES

Chylomicron counts were done in nine normals and twenty-six sprue patients concurrently with serum fat curves in which the total fatty acids, total cholesterol and lipid phosphorus, were estimated (BLACK and SIMPSON, 1947) A comparison of the chylomicron changes with the blood lipid changes during fat absorption might be expected to show whether chylomicrons correspond to any of the recognized fractions of the blood lipids

*The fasting values* of the chylomicron curves in the fat curve series fell into two groups thirty-one were under 30 and six were over 40 No significant difference was found in the fasting values for total fatty acids, phospholipids or total cholesterol in these two groups

*In the curves* the chylomicron counts were compared with the increments over the fasting value of each lipid fraction The peak of the chylomicron curve did not exactly correspond to the peak rise of any of the lipid fractions It was most closely associated with the peak rise in the neutral fat fraction, coinciding



with it in a third of the curves. In the remaining curves the chylomicron peak occurred about as often before as after the neutral fat peak. No significant association was found between the chylomicron peak value and changes in the phospholipid and cholesterol values. On the other hand, there was a significant correlation between the chylomicron peak value and the maximum neutral fat increment with values derived from the first thirty four curves, nine normal and the rest from sprue cases, the correlation coefficient was  $+0.38$  (standard error 0.17), which is statistically significant. As most of the total fatty acid increment after the 2nd hour consists of neutral fat, the chylomicron peak value may be expected also to correspond, though less closely to the total fatty acid peak value. For this association the correlation coefficient was found to be  $+0.33$ . Similar studies by other observers (MACARTHUR, 1930) have given comparable results.

Although there is a correlation between the chylomicron count and the neutral fat increment, it is not close. With absorbed fats, as with the fasting serum fats, the chylomicrons may represent only a variable fraction of the neutral fat. Table III gives the mean neutral fat increment over the fasting level

TABLE III.

MEAN NEUTRAL FAT INCREMENT IN SERA GROUPED ACCORDING TO CHYLOMICRON COUNT.

Chylomicron count.	Sprue.		Normal.	
	Mean neutral fat increment mg./L.	Number of specimens.	Mean neutral fat increment mg./L.	Number of specimens.
0—	0.48	23	0.19	18
40—	0.63	39	2.11	9
100—	1.36	25	1.86	11
240				

according to the height of the chylomicron count in sprue patients and in normals. For a similar increase in the chylomicron count there is on the whole a smaller neutral fat increment in sprue than in normals, which does suggest that the chylomicrons represent only a variable proportion of the absorbed neutral fat and that this proportion is greater in sprue patients than in normals. The fraction of the neutral fat represented by chylomicrons may be dealt with in a different way from the rest of the absorbed neutral fat. A rise in chylomicrons with a fall in the neutral fat values was sometimes observed in sprue patients. In eight out of eighty-seven specimens taken after the fat meal, the neutral fat values were lower than the fasting values. The average chylomicron count for these specimens was 69 representing a definite rise from the fasting level. These results suggest that invisible neutral fat is removed to the depots more rapidly than the particulate form so that the chylomicron count remains high although the neutral fat value falls.

In general, it may be concluded from the results of balance experiments and blood analysis that, while chylomicrons probably represent one form of absorbed fat, the changes in the count cannot be taken as an index of total fat absorption and give only a general indication of the amount of absorbed fat in the serum at the time of the count. The results now to be described in sprue patients give some further information on the significance of the chylomicron curve.

#### CHYLOMICRON COUNT IN SPRUE

Eighty-seven curves were done on twenty-eight patients with sprue. The time of the peak value of the curve was much more variable in sprue patients than in normals, and often delayed, the average time of the peak in normals was  $2\frac{1}{2}$  hours, in sprue patients later than 3 hours. A similar abnormality is frequently found in the glucose tolerance curve in sprue. The chylomicron peak was frequently low (Table IV). The occurrence of low curves has been

TABLE IV  
CHYLOMICRON PEAKS IN SPRUE GROUPED ACCORDING TO LIVER THERAPY AND DIARRHOEA.

ON PEAKS IN SPRUE GROUPED ACCORDING TO LIVER THERAPY AND DIARRHOEA.						
Peak of chylomicron curve	First curve on untreated patients without diarrhoea	All curves				
		Without liver therapy		With liver therapy		Total
		Without diarrhoea.	With diarrhoea	Without diarrhoea	With diarrhoea	
0-50	3					
51-100	9	8	11			
>100	11	18	2	0	4	23
		23	0	7	0	27
Total	23	49	13	13	1	37
				20	5	87

shown not to be simply related to the degree of steatorrhoea, on the other hand, low curves are usually found with diarrhoea, and in cases uncomplicated by diarrhoea low curves are much commoner in the untreated cases than in those receiving parenteral liver therapy (Table IV). The normal curves in the untreated cases were usually found in patients who were not very ill, though they often had marked steatorrhoea. The changes in the chylomicron count associated with liver therapy were studied in four cases with an initially low chylomicron curve and without diarrhoea. The results are shown in Table V. In each patient a normal count was found 2 or 3 weeks after beginning liver therapy (40 c.c. of a concentrated extract followed by 2 c.c. daily). In three of the patients the percentage of dietary fat which was being absorbed was known from the results of fat balance experiments and is shown in the last column of the table. In two of the patients the change in the chylomicron

TABLE V  
EFFECT OF LIVER THERAPY ON THE CHYLOMICRON CURVE IN SPRUE.

Patient.	Liver therapy date begun.	Chylo-micron curve date.	Time of specimen.				Percentage of dietary fat absorbed.
			Fasting.	$\frac{1}{2}$ hours.	2 hours.	$3\frac{1}{2}$ hours.	
N	5.6.45	4.6.45	11	47	49	41	
		6.6.45	2	30	53	66	
		12.6.45	10	33	37	77	
		27.6.45	21	65	100	123	
Ca	1.6.45	19.7.45	6	69	80	72	80
		6.6.45	20	70	120	99	83
Co	6.1.45	2.1.46	3	46	80	50	57
		24.1.46	2	120	100	50	82
Du	16.1.46	7.1.46	55	48	65	43	55
		24.1.46	12	40	120	56	86

curve was not associated with any significant change in total fat absorption in the third fat absorption had increased.

In the course of the investigation of absorption in sprue it was found that, as with the chylomicron curve, the height of the blood sugar curve was not simply related to the severity of the fat absorption defect. On the other hand, some association was found between abnormal chylomicron curves and abnormal sugar curves. Thirty five of the chylomicron curves were done within a week of a blood sugar curve following the ingestion of 50 grammes of glucose: the relation between the two series of curves is shown in Table VI. In about

TABLE VI  
ASSOCIATION BETWEEN HEIGHT OF CHYLOMICRON CURVE AND GLUCOSE CURVE IN SPRUE.  
(Cases with diarrhoea excluded.)

		Chylomicron peak value.		Total.
		>100	<100	
Maximum blood glucose increment before 1½ hours	>34 mg. per cent.	11	6	17
	<34 mg. per cent.	8	12	20
Total		19	18	37

two-thirds of the cases the findings in these two tests of absorption were in agreement

Thus, in sprue, the defect in absorption represented by a low chylomicron count shows a consistent response to liver therapy, unrelated to any change in total fat absorption, and it is associated with the deficiency in glucose absorption which is often found in this disease

## DISCUSSION

The results suggest that chylomicrons represent a fraction of absorbed fat which is dealt with differently from the greater part of absorbed fat. This conclusion is in accord with FRAZER'S (1940) partition theory of fat absorption. This theory has recently been discussed again at some length in another review (FRAZER, 1946). FRAZER claims that fat is absorbed in two ways in the normal subject, as split fat and as neutral fat which has not undergone any splitting by lipase and is absorbed in the particulate form (chylomicrons). FRAZER attaches some importance to the possibility of absorption of fat in this unsplit form. His theory has been applied to sprue by STANNUS (1942), who suggests that in sprue an important fraction of the dietary fat is normally absorbed, as neutral fat, and that the absorption defect only affects split fat.

The amount of fat normally absorbed without splitting is probably a small proportion of the whole (BLOOR, 1943, page 87). In our experiments with calcium salts the chylomicron rise during fat absorption was greatly diminished without producing any comparable increase in faecal fat. In fact it is known (BLOOR, 1943, page 29) that calcium salts diminish fat absorption, but the increased faecal loss of fat appears to be too small to be shown clearly in a 6-day balance experiment. The disproportionately low chylomicron counts found may be explained on FRAZER'S theory by assuming that the splitting of the fat in the bowel is more complete when a calcium salt is given with the meal. Lipase normally splits fat up to a certain equilibrium point. The formation of insoluble calcium soaps would remove split fat from the reaction mixture so that more neutral fat would split. Less neutral fat would be present in the bowel to be absorbed unchanged as chylomicrons. The amount of neutral fat that is normally absorbed as chylomicrons must be small since a decrease in absorption in this form did not result in an appreciable increase in the faecal fat content.

A defect in the absorption of total fat, shown by steatorrhoea, may be present in many cases of sprue in which normal absorption of unsplit fat is shown by a normal chylomicron curve. Defective absorption of unsplit fat, as shown by a low chylomicron curve, may occur particularly in the severe cases. Parenteral liver therapy corrects this abnormality, while total fat absorption is apparently often unaffected. This again suggests that the amount of fat normally absorbed without splitting is small, so that when split fat is not being absorbed the correction of any impairment in the absorption of unsplit fat alone would not be reflected in any detectable improvement in total fat absorption. Chylomicrons

TABLE V

EFFECT OF LIVER THERAPY ON THE CHYLOMICRON CURVE IN SPRUE

Patient.	Liver therapy date begun	Chylomicron curve date.	Time of specimen.				Percentage of dietary fat absorbed.
			Fasting.	2½ hours.	3 hours.	3½ hours.	
No	5.6.45	4.6.45	11	47	49	41	
		8.8.45	3	30	23	64	
		12.8.45	10	23	81	77	
		21.8.45	31	93	100	123	
Ca	1.8.46	18.7.45	8	29	29	72	87
		8.8.45	70	70	120	90	53
Co	6.1.45	3.1.46	2	40	50	80	87
		24.1.46	2	100	100	80	82
Ba	18.1.46	7.1.46	58	45	68	43	23
		21.1.46	13	40	120	50	26

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TABLE VI.

ASSOCIATION BETWEEN HEIGHT OF CHYLOMICRON CURVE AND GLUCOSE CURVE IN SPRUE.  
(Cases with diarrhea excluded.)

	Chylomicron peak value		Total.
	>100	<100	
Maximum blood glucose increment before 1½ hours	>24 mg. per cent.	8	17
	<24 mg. per cent.	13	18
Total	18	18	35

two-thirds of the cases the findings in these two tests of absorption were in agreement

Thus, in sprue, the defect in absorption represented by a low chylomicron count shows a consistent response to liver therapy, unrelated to any change in total fat absorption, and it is associated with the deficiency in glucose absorption which is often found in this disease

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A defect in the absorption of total fat, shown by steatorrhoea, may be present in many cases of sprue in which normal absorption of unsplit fat is shown by a normal chylomicron curve. Defective absorption of unsplit fat, as shown by a low chylomicron curve, may occur particularly in the severe cases. Parenteral liver therapy corrects this abnormality, while total fat absorption is apparently often unaffected. This again suggests that the amount of fat normally absorbed without splitting is small, so that when split fat is not being absorbed the correction of any impairment in the absorption of unsplit fat alone would not be reflected in any detectable improvement in total fat absorption. Chylomicrons

represent, however an appreciable proportion of the serum fats during fat absorption (ELKER, FRAZER and STEWART 1939), so that it must be assumed that the small amount of fat absorbed as chylomicrons is removed to the depots relatively slowly.

The different response to parenteral liver therapy in the absorption of unsplit and split fat suggests that a failure of different mechanisms is responsible for the deficiency in absorption of the two fractions in sprue. It appears that the fundamental deficiency is in the absorption of split fat, which may be due to a failure in phosphorylation (STANFORD, 1942). Less specific absorption defects, as of neutral fat, may be added later in the more severe cases.

### SUMMARY

Chylomicron curves were done on eleven normal subjects (eighteen curves) and on twenty-eight patients with sprue (eighty-seven curves). With a meal containing 18 grammes of fat, the peak count in normals ranged between 100 and 270 particles per standard field. The chylomicron curve in normals increased and diminished when more or less fat was given, suggesting that chylomicrons do represent at least one form of absorbed fat. On the other hand, low curves were produced in normals by giving calcium lactate with the meal in fat balance experiments, calcium lactate did not produce steatorrhoea, so the fraction of ingested fat involved in producing a normal chylomicron count must be quite small. Moreover in cases of sprue little relation was found between the height of the curve and the degree of steatorrhoea and when "flat" chylomicron curves returned to normal with clinical improvement, there was no corresponding diminution in the steatorrhoea. Concurrent chylomicron curves and serum lipide curves in sprue patients showed that chylomicrons represent a variable fraction of the absorbed neutral fat in the serum. In untreated sprue low curves were found in about half of the patients examined. After 2 or 3 weeks of parenteral liver therapy patients whose curves had been low gave normal curves.

It is concluded that a small proportion of the total fat is absorbed without splitting as finely emulsified neutral fat. While all patients with sprue have a defective absorption of split fat, only some show impaired neutral fat absorption as judged by the chylomicron count. Failure to absorb neutral fat is more common in the severe cases and responds to liver therapy.

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## GROWTH OF PROTOZOA IN TISSUE CULTURE V *LEISHMANIA DONOVANI*

BY

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The purpose of this paper is to describe the growth of *Leishmania donovani* in tissue culture using the technique described, in previous papers of this series, for *Plasmodium gallinaceum* and other parasites (HAWKING, 1945b, etc.) A preliminary notice of this work has previously appeared (HAWKING, 1945a)

### TECHNIQUE

The spleen was removed aseptically from hamsters infected with *L. donovani*. These animals, and the cultures used below, were kindly supplied by Mr L G GOODWIN, of the Wellcome Research Institution. The strains of parasites had been isolated from cases of kala-azar in India, Sudan, and Sicily, no significant difference was observed in their behaviour in these experiments. The spleen was cut up into small pieces. Four small pieces of cover slip were attached to the floor of a Carrell flask by plasma, and an explant of spleen was mounted on top of each in a very small drop of fowl plasma. The fluid medium consisted of serum, 20 per cent, chick embryo extract, 5 to 20 per cent, phenol red, enough to give a faint red colour, and Tyrode's solution (HAWKING, 1945b). In the earlier experiments, hamster serum was used. In the later experiments rabbit serum was employed, it seemed as favourable for growth as hamster serum and was much easier to obtain. The medium was changed about every 5 days or whenever acidity was indicated by the medium turning yellow. The growth of cells and parasites was examined by removing one of the glass slips, fixing in Schaudinn, staining with Giemsa, differentiating with acid, dehydrating with acetone and xylol, and mounting in a neutral mountant. In some of the experiments, the explants were made from the spleen of an uninfected hamster, after a few days a drop of a culture of *L. donovani* containing flagellate forms was added.

\* Grateful acknowledgements are due to Mr L G GOODWIN for cultures of leishmania, and for infected hamsters, to Miss V D MARKHAM for technical assistance, to Mr F V WELSH, and Mr C D SUTTON for the photography, and to Miss M M BURCHELL for assistance with the illustrations.



## EXPERIMENTAL RESULTS.

Most of the cultures made from infected hamsters grew well, and after a few days leishmania could be seen in the macrophages migrating out from the explant. In smears made from the spleens used for these cultures the number of parasites was such that most cells contained about one to three leishmania. In the cells of the cultures they became much more numerous, until up to 200 or more were present, as shown in Figs. 16 and 18. Cultures were maintained up to 33 days, termination being due either to exhaustion (by removal for examination) of all the cover slips in a flask or to bacterial contamination.

## DEVELOPMENT OF LEISHMANIA IN CELLS.

In the first few days after the cultures were set up there was an active emigration of monocytes and other cells from the explant on to the adjacent surface—most of the cell types present in the original tissue were seen. Later (5 to 20 days) there was usually a mass of elongated cells (fibroblasts) around a circular space—the inner cells were arranged circumferentially the outer ones radially. Monocytes and macrophages were scattered among these cells, and many also occurred in the central space as very thin plate like cells flattened against the glass. In this position, giant cells containing five to fifteen (or more) circular nuclei were often present. Most of the cell types of spleen, other than those mentioned, had disappeared by the 8th day. After the 20th day the structure of the culture usually became more disorganized and cells growing singly or in small clusters might be found without any definite arrangement.

In the early stages, e.g., 2nd day leishmania were found in the monocytes migrating out from the explant. The cells contained two to five parasites or more, and did not seem to be incommoded by their presence. Later parasites were present in considerable numbers in monocytes and macrophages throughout most of the culture—sometimes distribution was a little patchy (Figs. 8, 9, 16 to 18, 22). They were especially numerous in the large giant cells mentioned above, Fig. 22. Sometimes a few parasites were found in cells which were definitely fibroblasts, Fig. 17. In the monocytes they tended to occur towards the edge of the cell—in the giant cells, similarly they congregated especially in the thinner peripheral portion. Some of the macrophages had long processes, and these often contained parasites arranged like a string of beads, Fig. 9. Parasites were sometimes seen lying free from cells, but such parasites were usually degenerate. In one slide a circle of parasites was found lying between the glass slip and an overlying layer of fibroblasts—presumably a large macrophage had degenerated, leaving the parasites in an apparently healthy condition.

The parasites in these cultures occurred mostly in the typical leishmania form, as shown in Figs. 1 and 2. That is, the parasites formed spherical or slightly oval bodies, with a rounded nucleus lying to one side and a square or oblong parabasal body. Occasionally the parabasal body was duplicated. Forms showing binary fission of the nucleus could be found only by long search.

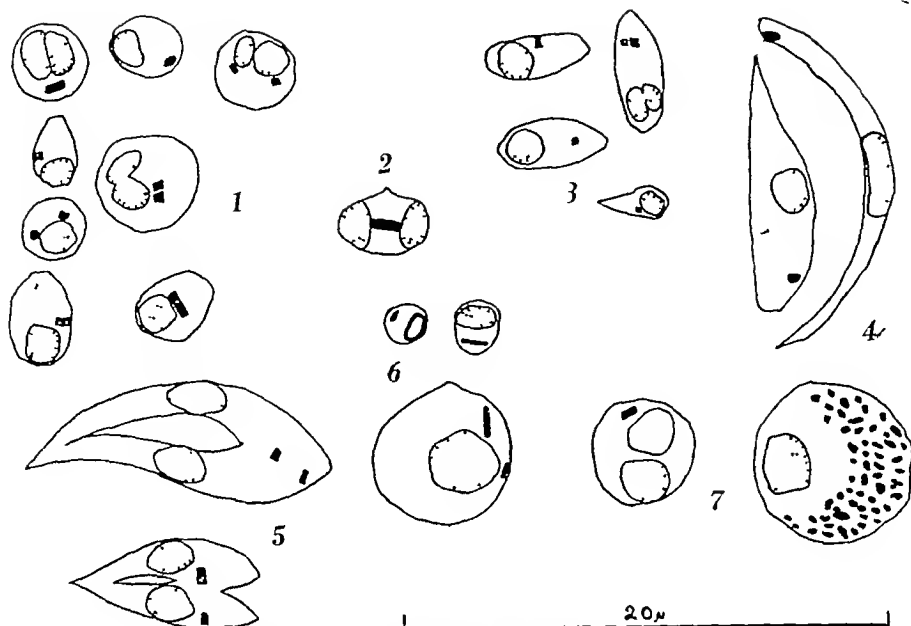


FIG 1—Typical intracellular leishmania in tissue culture  
 FIG 2—Dividing form from a fibroblast, unusual type (9th day)  
 FIG 3—Elongated intracellular parasites (12th day)  
 FIG 4—Leptomonad forms derived from intracellular leishmania, flagella not shown (19th day)  
 FIG 5—Dividing leptomonad forms derived from intracellular leishmania  
 FIG 6—Round extracellular forms derived from intracellular leishmania (19th day)  
 FIG 7—Degenerate intracellular forms (23rd day)

(FIGS 1-7—Magnification according to scale Drawn by F H)

In some of the cultures, leishmania could be found which were definitely elongated as though preparatory to forming flagellates (Fig 3). The circumstances favouring this were not clear. Definite elongation was seen in cultures 9 days old and 12 days old respectively, and in cultures 19 to 20 days old, definite formation of flagellates occurred as described below. On the other hand, cultures 24 days old might show only rounded parasites.

#### FORMATION OF FLAGELLATES FROM LEISHMANIA AT 37° C

In most of the cultures that were maintained after about 16 days, further development of the leishmania took place, in that they were liberated from the cells and formed great masses of extracellular flagellates. This phenomenon occurred in four out of six flasks which were maintained for more than 20 days, and it was observed in two experiments separated by the interval of a year. In two flasks forming the third experiment, the cells were rather degenerate by the 20th day and flagellates were not formed. In two other flasks, growth of leishmania was maintained in tissue culture for 24 and 30 days respectively,

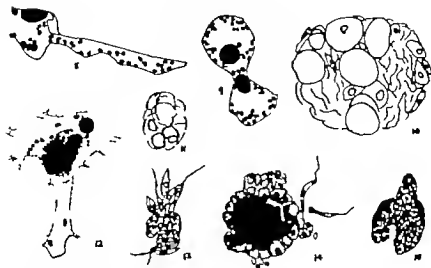


FIG. 8.—*Leishmania* on the process of cell (10th day).

FIG. 9.—*Leishmania* in two cells which have not yet completed their division (same slide).

FIG. 10.—Degenerate cell mass after parasites have escaped (same slide).

FIG. 11.—Similar form, more degenerate (same slide).

FIG. 12.—Intracellular *leishmania* 10 days after adding flagellates to culture of hamster cells.

FIG. 13.—Mass of flagellates adherent to each other from culture of infected hamster spleen (20th day).

FIGS. 14 & 15.—Dense masses of extracellular parasites (same slide as 12).

(Figs. 8-15. 60x. Drawn by M.M.B. V.D.M. and F.H. from photographs.)

the parasites being transferred from a culture of infected hamster spleen to a culture of uninfected spleen during this time (see below) in this experiment, no flagellates were formed. The stimulus responsible for this development is not clear. In view of the well known formation of flagellates by cultivation at temperatures below 30° C., consideration was given to the possible influence of temperature. These cultures had been kept in a constant temperature room at 37° C. but every 3rd to 4th day they were taken into the laboratory for examination and were kept at 16 to 20° C. for about an hour. To test the hypothesis that formation of flagellates was due to a lowered temperature, one flask was incubated at 30° C. from the 10th day onwards. Cells and parasites grew quite well at this temperature. Flagellates were absent from it at 3 p.m. on the 18th day but were present as great clusters on the morning of the 19th. In two similar flasks of the same experiment, which remained at 37° C. one contained no flagellates on the 15th day but great clusters of them on the 18th day and the other contained great clusters of round *leishmania* almost beginning to be liberated from cells on the 15th and 18th days. A comparison of these flasks, although not conclusive, suggests that a lowered temperature has not been the main stimulus for the conversion into flagellates.

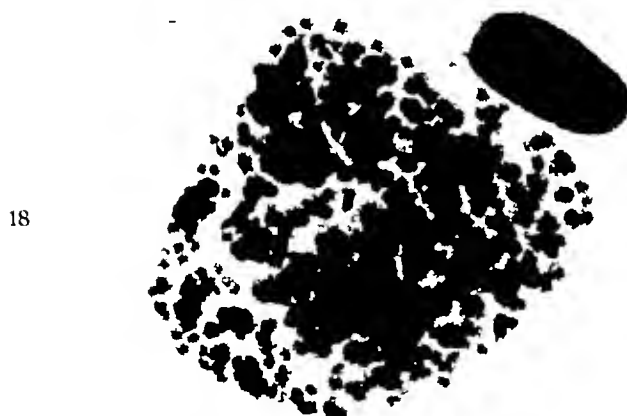


FIG 16 —Macrophage containing many leishmania, some of which have become elongated, 19th day of cultivation from spleen of infected hamster  $\times 1,250$

FIG 17 —Fibroblast containing leishmania, same slide  $\times 900$

FIG 18 —Macrophage greatly distended with leishmania, some of the leishmania lie in groups in the cytoplasm, which is reduced to a degenerate meshwork, same slide  $\times 1,250$

19

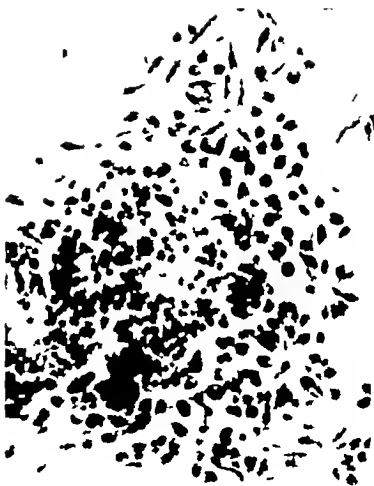


FIG 19.—Group of parasites ranging from leishmanias to elongated flagellates, released from disintegrated cell same slide 1,250



FIG 20 —Low power photomicrograph ( $\times 62$ ) showing dense masses of flagellates (the fine black dots) liberated from cells, the nuclei of fibroblasts and surviving macrophages are seen as small black masses, same slide

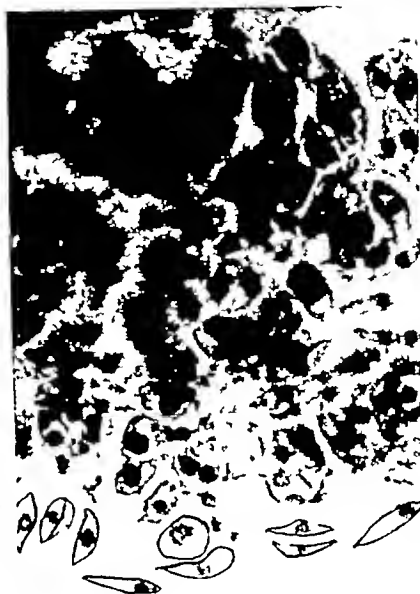


FIG 21 —Edge of dense mass of parasites multiplying extracellularly at  $37^{\circ}\text{C}$ , 19 days after flagellates had been added to cultures of hamster tissues (Some flagellates have been outlined in ink)  
 $\times 1,250$

13



23

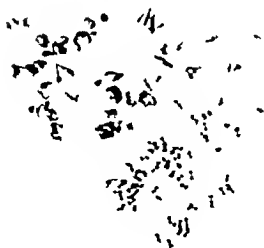


FIG 22.—Multinucleated cell containing leishmaniasis—  
culture from infected hamster. 5th day. 1000

FIG 23.—Group of parasites liberated from cell.  
1000

The various stages in the development of the flagellates are shown by the illustrations (Figs 18 to 20). Apparently the cells become distended with such large numbers of rounded leishmania that the nucleus atrophies and little remains except a bag (the cell membrane) full of parasites (Fig 18). At this point some of the parasites seem to form sub-groups of six to ten bodies contained in a vacuole formed by the remnants of the cell cytoplasm. The cell membrane breaks down liberating the rounded parasite which then assumes the elongated flagellate form (Fig 19). Multiplication seems to continue at all stages of this metamorphosis and soon enormous masses of flagellates are formed (Fig 20). These are visible to the naked eye as whitish masses. The flagellates are actively motile. In some cells, however, as was noted in a previous paragraph, certain parasites could be seen to be elongated at an earlier stage of development (Fig 16). One culture was maintained until the 32nd day, at which point it became infected with cocci. At this point, practically all the macrophages had been destroyed by the leishmania, the parasites were present as flagellates which formed enormous masses, and degeneration of the parasites seemed to be due to the bacterial contamination rather than to any intrinsic cause. In the fluid bathing the cultures, small rosettes of flagellates occurred (Fig 23). At one time or another the cultures seemed to contain all the seven types of parasites distinguished by CHRISTOPHERS, SHORTT and BARRAUD (1926). In addition, various abnormal forms occurring in these cultures may be noted —

- (1) Some of the parasites were greatly swollen, being circular in shape and up to  $7\mu$  or more in diameter, their cytoplasm often contained numerous small black or deeply staining granules (Fig 7). Similar granules were seen in many parasites which were only slightly unhealthy. Presumably these are degenerative changes.
- (2) There were large oval masses, measuring between  $25-60\mu$ . Towards one end there were usually several crinkled red disks (as stained with Giemsa) about  $4-5\mu$  across (presumably degenerated and shrunken cell nuclei). The rest of the mass consisted of shrunken bags with red-staining walls. In the less degenerate specimens some of these bags seemed to contain swollen parasites or groups of parasites (Fig 10). In the more extreme cases there was nothing in the bags and the whole structure was merely a honey-combed husk enclosing empty spaces (Fig 11). It is not clear whether disappearance of the leishmania was due to emigration or degeneration.
- (3) There were also masses of leishmania of various shapes, closely adherent to each other. These will be described further below.

### TRANSMISSION OF LEISHMANIA BETWEEN CULTURES

The attempt was made to transmit the infection from a tissue culture containing rounded leishmania to fresh cultures of clean hamster spleen. Three Carrel flasks were set up, each containing one glass slip with a culture of spleen plus leishmania (set up 8 days previously, both cells and leishmania growing well) and three slips with implants of fresh hamster spleen. The flasks were incubated in a stationary position as usual, but during the examinations which were made every 2 to 4 days, the fluid medium was rocked to and fro to mix it up. A slip of the new tissue was removed from one flask after 7 days and showed a small group of cells on the edge of the colony containing many



leishmania the rest of the colony contained hardly any parasites. The other slaps were examined on the 12th and 24th days and all showed leishmania to be present in the cultures of clean tissue as well as in the original infected cultures. (After the 12th day the flasks became contaminated with bacilli, which gradually caused degeneration of the cells and parasites.) Most of the slaps showed a few rounded leishmania lying on the glass and plasma often at some distance from the cells since no trauma had been incurred in removing the slaps from the flask and fixing them the extracellular position of these parasites probably represents the true state of affairs during cultivation rather than an artefact produced when the preparation was made. The occurrence of such extracellular forms would explain the ready transmission of leishmania from one cell colony to another. No evidence of metamorphosis of the parasites into the leptomonad form during this transmission to other cell colonies was seen.

### *Infection of Cells by Flagellates.*

In a further series of experiments, flagellates obtained from cultures made at 25° C. were added to flasks containing implants of clean hamster spleen, which had already grown *in vitro* for 3 or 4 days. In most of the cultures rounded leishmania were found inside the cells when the flasks were sampled (Fig. 12). At the 2nd and 4th days, the leishmania were few by the 8th, 10th, 13th and 16th days they were often numerous and in some places they distended the cells as in the cultures made from infected spleen described above. The forms in the cells were usually rounded, but sometimes they were elongated. Their distribution was often uneven, being numerous in some parts of the cell colony and absent in others. In one culture, cells were seen stuffed with flagellates, with the flagella sticking out round the edge of the cells.

### MULTIPLICATION OF FLAGELLATES AT 37° C.

In many of the cultures the flagellates actively multiplied in the fluid medium of the cultures, at 37° C. quite independently of the cells. The possibility of such growth *in vitro* at 37° C. has not previously received general recognition. Numerous flagellates with the usual leptomonad form and actively waving flagella were found in the fluid after 11 days or more of incubation at this temperature. Such growth occurred vigorously in many flasks but it was absent in others it happened in several different experiments. Its presence or absence could not readily be associated with any particular variation of the culture medium or any particular strain of parasites. Usually the multiplication took place by the usual binary fission of leptomonad forms, and often rosettes were produced.

Sometimes there were curious formations of parasites closely adherent together these formations deserve further description. They were most

common in the flasks where cultures of flagellates had been added to colonies of fresh spleen tissue, but they were also found in the flasks described above in which flagellates had broken out from the cells containing rounded leishmania. For the purpose of description, three types might be recognized, although they merged into one another, it was not clear whether or not they were three stages of the same process.

(1) The smaller groups consisted of four or five elongated flagellates, lying closely side by side with their flagella entwined together at one end, as though a parasite had undergone longitudinal division several times, but the resultant daughter-forms had remained tightly adherent to each other, e.g., the four end parasites in Fig 13.

(2) The medium groups consisted of ten or more flagellates, which were still adherent to each other but some had been displaced in a longitudinal direction so that the nuclei no longer lay opposite one another (remainder of Fig 13).

(3) Finally, there were large masses which were too deeply stained for the internal structure to be easily visible, but the outer parts seemed to be composed of flagellates as in the smaller groups. The formations were mostly oval in shape, often measuring up to  $40\mu$  or more in length (Fig 14), sometimes the outline was more complicated as in Fig 15. The parasites were mostly pyriform, with some distortion, due to mutual compression. They were closely adherent one to the other, except towards the edges of the mass where incipient cleavage could often be detected. Apparently no flagella were formed until after cleavage had occurred. It was difficult to make out whether or not there was an external membrane enclosing these masses of parasites and holding them together, probably there was no such membrane. In some groups, there were one or more degenerate pyriform parasites adherent to the outside of the general mass as though formed by budding from the other parasites, this appearance is also evidence against an external membrane. Fig 21 (which was taken from the edge of a large mass of parasites) apparently represents one of these formations breaking down. These masses usually occurred at a considerable distance away from the cells, with which they seemed to have little or no association. There was no suggestion that they originated as intracellular formations, in which the cell was later destroyed. The environmental conditions which favour their production are not clearly known.

## DISCUSSION

This investigation was undertaken primarily to explore the potentialities of the tissue culture technique originally described for the cultivation of the erythrocytic forms of *P. gallinaceum*. However, the results raise interesting questions about certain aspects of the development of leishmania.

Previous reports on the cultivation of leishmania inside living cells are as follows.

GAVERILOV and LAURENCIN (1938) described experiments with a tissue culture technique, using cover glass preparations. Their account is often difficult to follow, and many of their attempts were unsuccessful. They cultivated the tissue of hamsters infected with leishmania and, apparently, demonstrated the parasites in the cells after several days *in vitro*. In their last experiment they added cultures of the flagellate forms to cultures of embryonic liver and demonstrated leishmania in the cells up to 9 days later. They also placed an implant of clean embryo spleen side by side with an implant of infected hamster spleen and found that 7 days later leishmania were present in cells of the clean implant. In many of their experiments leishmania formed large extracellular colonies in the plasma clot which constituted the medium

TECHEROMORITZ (1946), in the English summary of a Hebrew paper reports that he obtained pure cultures of leishmania of *L. infantum* and *L. donovani* by planting flagellates on tissue cultures. Apparently he used hamster spleen and plasma and serum from rabbits or cattle.

WEINMAN (1936) observed growth of leishmanial forms when the flagellates of *L. tropica* were added to guineapig tissue, surviving on agar-serum-tyrode slants at 37° C. but no growth occurred in true tissue cultures of guineapig tissue.

The technique described in the present paper appears to yield intracellular cultures of leishmania more conveniently and consistently than those reviewed above, with the possible exception of TECHEROMORITZ's methods for which details are not available. The cultures are particularly well adapted for microscopical study.

The intracellular growth of the parasites in the cultures made from infected hamsters was in accordance with expectations based on previous experience with tissue cultures of *P. gallinaceum* and *T. cruzi*, and on reports in the literature. Two phenomena were unexpected, viz. —

(i) The flagellates bursting out of the cells after 16 days, although the temperature was maintained at 37° C.

The stimulus responsible for this is not clear. It did not occur until after 16 days and happened only with cells which were greatly distended with the parasites. The single experiment made by incubating one flask at 30° C. did not support the view that the stimulus might be lowering of the temperature. It is probable that something of this nature was seen in one of the experiments of GAVRILOV and LAURENCE. The only other record found of similar formation of flagellates at body temperature is the observation of WIERROCK (1936) of leptomonal forms in the spleen of a dog experimentally infected with *L. donovani*. In the experiment of the present series in which the infection passed from infected cultures to clean cultures no such leptomonal forms were seen, but rounded leishmania occurred extracellularly. Accordingly there is no need to postulate transformation into the leptomonal form to explain transmission from one cell to another in the vertebrate host; more probably transference occurs via the leishmanial form.

(ii) Multiplication of flagellates at 37° C.

As described above extracellular multiplication of flagellates occurred actively at 37° C. both in flasks where flagellates had been added and in those in which flagellates burst out of the cells. Apparently the same phenomenon was observed in some of the cultures of GAVRILOV and LAURENCE, and of WEINMAN; but the possibility of such growth of the leptomonal form at body temperature has not been generally recognized. At present, we do not clearly know what conditions definitely determine which form the parasite shall take at given moment. The leishmanial form is favoured by temperature of 37° C., by intracellular position and apparently by immune serum (ADLER and TAYLOR, 1926) and the leptomonal form by temperature of 30° C. or less and by media of the N.N.N. type. But none of these factors explains all the results described above. The subject deserves further study.

The curious masses of parasites which were observed when the flagellates multiplied at 37° C. were very striking (Figs. 14 and 15). DAS GUPTA (1922) mentions somewhat similar forms (his Fig. 5), but he considered that a cyst was present. GAVRILOV and LAURENCE (their photograph No. 13) depict a

similar colony which they had observed when cultures of leishmania were added to hamster plasma and embryo juice and grown at 37° C, they also interpreted the appearances as a "cystic colony". In the present instance no cyst walls could be distinguished. Apparently these masses were formed by the continued mutual adhesion after fission of pyriform parasites which were intermediate between the leishmanial and the elongated leptomonad stage. In many ways they resemble the plug of parasites which develops in the pharynx of an infected sandfly, as described by SHORTT, BARRAUD and CRAIGHEAD (1926).

This work confirms the view expressed in previous papers of this series that many kinds of protozoal parasites, growing in association with cells amenable to tissue culture, can be cultivated by the present technique. When dealing with small animals—e.g., hamsters, the preparation of the fluid part of the medium is much facilitated if a suitable heterologous serum obtained from a larger animal—e.g., rabbit, is used. These cultures should be convenient for chemotherapeutic investigations of anti-leishmanial compounds since parasite, tissue cells, and drug could all be combined in a small flask and the effects of the drug on the parasite and on the cells respectively could be conveniently ascertained within a few days. Chemotherapeutic investigations of this kind on exoerythrocytic forms of *P. gallinaceum* growing in tissue culture, have been described from this laboratory by TONKIN (1946).

The protozoa which have been grown in tissue culture by this technique now include the exoerythrocytic forms of *P. gallinaceum* (HAWKING, 1945b), of *P. relictum* (HAWKING, 1946) and of *P. lophurae* (TONKIN and HAWKING, 1947), *Trypanosoma cruzi* (HAWKING, 1946), and *L. donovani*. Attempts have been made to culture *Haemoproteus columbae*. Two infected pigeons and pupae of *Pseudolynchia maura*, were kindly supplied by Dr R. COATNEY, of the National Institute of Health, Bethesda, Md., U.S.A. The pigeons and flies were kept in the cages described by HUFF (1937), and transmission occurred to about twenty other pigeons. However, the strain of *Pseudolynchia* tended to die off in the winter, possibly because the air became too dry for them. Suitable pigeons were killed and portions of the lung were set up for tissue culture by the usual technique. About half the flasks became infected with moulds or other organisms. In the other half, growth of macrophages and similar cells occurred but no parasites could be found. It is considered that this failure of growth was probably due to the difficulty of securing implants of lung tissue which contained the asexual forms of *Haemoproteus*. Owing to the pressure of other work, this line of research was discontinued.

### SUMMARY

*Leishmania donovani* has been grown in tissue culture at 37° C, using the technique previously described for the exoerythrocytic forms of *P. gallinaceum*. The cultures were made from the spleen of infected hamsters, but rabbit serum was substituted for hamster serum in the fluid phase of the medium, because it was more easily obtainable. Vigorous growth of leishmania occurred and

growth was maintained as long as 32 days. Infection could easily be transmitted to cultures of clean spleen grown in the same Carrell flask as an infected culture.

In most of the cultures maintained for more than 16 days, the parasites burst out of the cells and formed great masses of free flagellates, growing and multiplying at 37° C.

During other experiments, flagellates from cultures made at 25° C. were added to tissue cultures from hamster spleen growing at 37° C. Some of the parasites entered the cells assuming the leishmanial form others multiplied as flagellates in the fluid medium at 37° C.

Among the flagellates growing at 37° C. peculiar colonies were found consisting of pyriform or polygonal parasites closely adherent to each other

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## STUDIES ON LIVER DAMAGE IN ACUTE MALARIA

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In 1931 TSCHILOW, and in 1944 KLEEGER, found by means of the Weltmann Reaction (WR) that blood serum of patients suffering from acute malaria coagulates at a lower concentration of calcium chloride than serum of normal individuals, or of patients suffering from an acute inflammatory disease. This method was originally evolved in order to distinguish acute exudative inflammatory from chronic proliferative processes. Essentially, the serocoagulation test consists in mixing constant amounts (0.1 c.c.) of serum with constant amounts (5.0 c.c.) of calcium chloride at varying concentration and heating the mixture in a boiling water bath. Normal sera, when heated, will coagulate at a calcium chloride concentration of 0.05 to 0.04 per cent, i.e., the sixth and seventh tube of the dilution series. In exudative inflammatory processes, especially in acute infections, the coagulation band is shifted as a rule to the left. Results obtained with the reaction in malaria by both authors were surprising, since the clinical picture in such cases is that of an acute infection with high fever where one would expect a typical shift to the left. According to WELTMANN, the coagulation band tends to be shifted to the right in the following conditions: (a) toxic liver damage, (b) haemolysis, (c) chronic proliferative processes, especially liver cirrhosis. KLEEGER, in his first paper on acute malaria, has discussed the possible role of the first two factors in the production of the lengthened coagulation band.

\* We wish to thank Dr F ROSENTHAL, who made the cholesterol determinations for us.

In order to elucidate factors which influence the Weltmann Reaction in acute malaria, and hoping thereby to define indications for suitable therapeutic or dietetic measures, a more extensive experiment was undertaken. The number of patients suffering from naturally acquired *Plasmodium vivax* and *P. falciparum* malaria was increased to about 100. To the Weltmann Reaction, red cell and reticulocyte counts, haemolysis, Cephalin-cholesterol Flocculation Test (C.F.T.), Chornie Test and cholesterol examinations were added.

### MATERIAL AND METHODS.

Blood was withdrawn by dry sterile syringe in order to avoid haemolysis, even traces of which are likely to shift serum coagulation to the right by 1-2 numbers. Sera were obtained from Arab patients, 15 to 60 years of age in hyperendemic villages (about per cent. of cases), or from people who had acquired the infection during visit to malarial areas but who lived permanently in non-malarial districts. Sera were also obtained from Jewish patients kept under strict medical supervision and from groups of normal adult Arabs living in hyperendemic area.

The Weltmann Reaction was performed according to the directions given in the *Laboratory Manual* by LEVISON and MCFATE, or by GRAYSON. In the haemolysis test, introduced by MAR, BIRKBAUM and KLIGER, solution of dried ox bile in isotonic saline served standard haemolytic solution which takes normal blood in 4.5 to 8 hours. The lysis of blood becomes enhanced in the course of malarial infection. This test was performed concurrently with reticulocyte and red cell counts.

In the Cephalin-cholesterol Flocculation Test the directions by HANCOCK were followed. It should be pointed out that there were no hospital facilities, the material being obtained from patients attending field clinic of the Malaria Research Station. Under field conditions re-examinations involved considerable difficulty. Owing to lack of proper facilities, we were compelled to perform the cholesterol-ester estimations in Jerusalem. A modification in the procedure was, therefore, introduced. 0.7 c.c. serum was mixed with 37.5 c.c. ether-alcohol (1:4), filtered, washed and evaporated. The distillate was mailed to the laboratory where the dry residue was redissolved and titration carried out.

### RESULTS AND DISCUSSION

Graphs I and II summarize the results of tests made on forty and fifty-six patients suffering from acute *P. vivax* malaria and *P. falciparum* infection respectively.

The blood of patients of Weltmann groups 6 and 7 is laked in the standard bile solution in B.T. malaria after 3.1 and 3 hours, and in M.T. malaria after 3 and 3.2 hours. Normal blood in the same haemolytic solution was invariably laked after 4.5 to 5 hours. The mean values of haemolysis in Weltmann groups 7 and 8 in cases of *P. vivax* and *P. falciparum* malaria were found to differ significantly the difference being more than three times the standard error of the difference between both means. The difference between the means of haemolysis time of Weltmann groups 8 and 9 was significant only in the case of the group of patients suffering from *P. vivax* malaria, in which it was six times the standard error of difference. In corresponding groups with *P. falciparum* infection the difference of the means was less than twice the standard error of the difference.

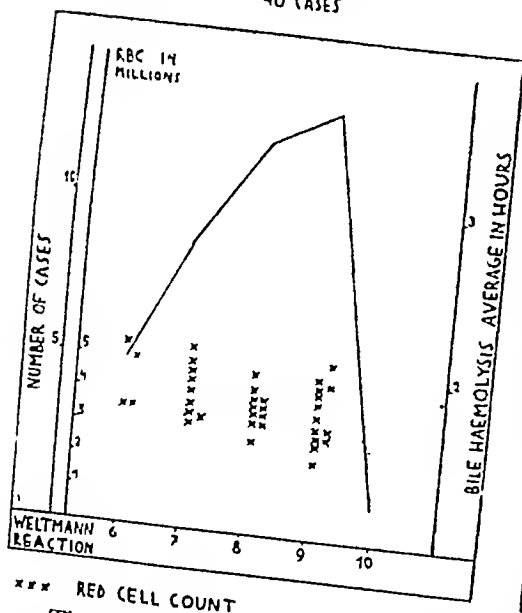
Red cell counts (indicated by "crosses" in the graphs) tend to decrease in number (below 4,500,000/mm) in the groups with a pathological Weltmann Reaction. All anaemic malaria patients show a pathological Weltmann coagulation band (figures of 8 or 9). There are, however, cases with right shifted WR without anaemia. The decrease in the number of red cells appears to be correlated to an increased lytic tendency in the blood.

The average reticuloocyte values in the Weltmann groups 8 and 9 exceeded 20 per mille.

Results in Graphs I and II refer to patients who had not previously received anti-malarial treatment. The findings suggest that globulin alterations indicated by the abnormal Weltmann Reaction are brought about by malarial infection.

GRAPH I

P. VIVAX MALARIA BEFORE TREATMENT  
40 CASES

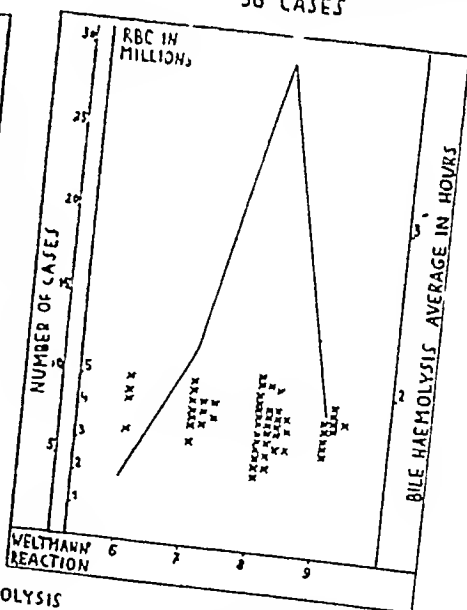


\*\*\* RED CELL COUNT

BILE HAEMOLYSIS

GRAPH II

P. FALCIPARUM MALARIA BEFORE TREATMENT  
56 CASES



— WELTMANN REACTION

The full-drawn line indicates the distribution of sera coagulating in the sixth to tenth tube, i.e., at 0.05 to 0.01 per cent of calcium chloride (WELTMANN). The dotted line gives the mean haemolysis time in hours for each group of patients, classified according to the calcium chloride dilution at which the serum coagulates.

It seemed of special interest to study the effect of anti-malarial drugs on this reaction. Eleven B.T. and ten M.T. malaria cases were examined prior to a short-term treatment with quinine or atabrin, and again one to three times during a period of from 4 to 30 days after the attack.



From Table I it appears that the changes in reaction are small neither quinine nor mepacrine alter the test. This has been clearly demonstrated also on a group of ten patients suffering from B T relapsing malaria. Sera of these patients were tested before and after treatment, the observations covering in

TABLE I

	B T cases.				10 ALT cases.			
	Weltmann.				Weltmann.			
	3	7	2	2	6	7	2	9
Before treatment—number of cases	1	7	2	1	2	2	4	2
Tested 1-3 times during 4-30 days after attack—number of cases	1		3		2	2	3	1

some instances periods of 2 to 6 months. The diagnosis was established 24 to 46 hours after the first attack. Patients in this group were given careful medical supervision. Within 12 months they had from two to eight relapses, each of which was promptly treated with mepacrine or quinine, the treatment being continued for 7 to 10 days under strict supervision. Results obtained on this group were of a high degree of constancy in repeatedly performed tests.

TABLE II

	Weltmann			
	3	2	7	2
Number of cases	1	1	4	1

The results differ from those obtained in other patients with *P. vivax* malaria (Graph I), since they reveal the pathological shift to the right in a smaller proportion of cases.

The question whether there is any significant difference in the pathological alteration of the Weltmann Reaction in *P. vivax* and *P. falciparum* malaria respectively is not in our opinion, conclusively answered by our results, although the differences observed are apparently not inconsiderable.

This may be explained by the fact that the therapeutic measures were taken early and methodically whereas the other group of patients lived under unfavourable conditions, especially with regard to medical services.

TABLE III

	Weltmann				
	6	7	8	9	
<i>P. vivax</i> malaria	13	23	31	33	per cent
<i>P. falciparum</i> malaria	7	20	56	17	"

Another point to bear in mind is offered by the results of the liver and spleen palpation in ninety-five malaria patients which are summarized in Table IV

TABLE IV

	Weltmann			
	6	7	8	9
Total number of patients	9	21	42	23
Patients with palpable or enlarged spleen	6	17	36	21
" " liver	2	5	19	14

Four cases of splenomegaly does not seem to influence the WR. One of the sera coagulated in the fifth, one in the seventh, and two in the eighth tube. Special mention should be made of two M T malaria cases with Weltmann 5. They are not included in Graph I because an acute infection of another kind most probably

affected the results of the Weltmann Reaction (active pulmonary tuberculosis in the one, bacillary dysentery in the second case).

Healthy adults with no previous history of malaria were used as controls in each series of tests. They all had normal figures (6 to 7).

An investigation conducted on healthy adults in a hyperendemic area was also carried out in order to find an answer to the question whether changes occur in the Weltmann Reaction in individuals who have acquired a natural resistance to malaria practically without treatment as a result of numerous infections during childhood. Adults of this category had not experienced any acute malarial attack during several years, and their blood examinations in thick drops on the day of examination were negative.

TABLE V

	Weltmann.		
	6	7	8
Number of cases	4	7	8

The results given in Table V are interesting with regard to their relation to liver damage. It should be mentioned that the five "normal" adults with Weltmann 8 were anaemic (averaged 3 100 000 as against 4,350,000 in the other eleven adults) and blood from them laked in bile solution more readily (2.6 hours as against 3.9 hours in adults with Weltmann 6 and 7). The five adults showed enlargement of the spleen (2 to 8 fingers below the costal margin). In four of them the liver also was enlarged. The sera of persons with palpable or enlarged spleens coagulated in the sixth and seventh tube. In this group, and in patients from hyperendemic areas, the possibility that chronic proliferative processes—especially of liver—due to numerous malaria infections may have contributed to the broadening of the coagulation band, cannot be excluded. It is, however, obvious that the five adults with Weltmann 6 were anaemic, and that their blood was definitely more sensitive to lysis.

By way of comparison between the Weltmann Reaction and other liver function tests, Table VI shows results obtained in eleven B.T. and five M.T. cases by the Weltmann Reaction, and the Cephalin-cholesterol Flocculation Test (C.F.T.), a method which has been widely used to demonstrate liver damage in malaria (MIRSKY *et al.*, 1944 BRONSTEIN and REID 1945 GUTTMAN *et al.* 1945 FREDRICKS and HORRAUER, 1945). It is clearly seen that the results do not run parallel, a prolonged coagulation band even being compatible with a negative result in the C.F.T. Therefore, no conclusive answer with regard to the extent to which the change in the Weltmann Reaction is due to liver damage can be expected from a comparison of both tests.

TABLE VI

Weltmann Reaction	Total number of cases	Cephalin-cholesterol Flocculation				
		O	+	++	+++	++++
5	1	1				
6 } 7 }	4	2		2		
8	3	1		1	1	
9	8	1	1	5		1

For many years it has been known that acute infectious diseases with high fever show a marked drop of serum cholesterol, values being re-established at the time of recovery (BOYD, 1935) FAIRLEY and BROMFIELD (1936), who used the method of MYERS and WARDELL, found at the height of malaria infection a hypocholesteremia of 113-129 mg per cent. Their figures for normal people in tropical countries range from 120-200 mg per cent. DUFOR found marked fall of total cholesterol in several cases and a lesser fall in mild cases of malaria. ROSS, WHITMORE and ROE do not confirm this observation (CIT by FAIRLEY).

The liver plays an important role in lipid metabolism, but estimation of total cholesterol alone does not reflect metabolic capacity. THANNHAUSER, about 20 years ago, demonstrated that the esterification of cholesterol takes place in the liver by means of an esterase, and that this esterification is reduced in cases of liver damage. Change in the normal and constant ratio of free to combined cholesterol in healthy persons is therefore particularly significant in the detection of liver damage.

In our malaria patients we examined the free and combined cholesterol, according to the method of ALLAN KAYE (1940). Our figures in normal adults, as reported in a study in typhoid fever (KLEEGER and ROSENTHAL) are very similar to those reported by KAYE in subjects from North America.

At the age of 20 to 30, an average of 160 mg per cent (range 125 to 230), and at the age of 30 to 40 an average of 175 mg per cent (range 140 to 240), were found for total cholesterol, and the ratio of free to esterified cholesterol was 1.26 to 1.3.

The normal values of cholesterol vary much according to the method of determination. We confine our own discussion to the ALLAN KAYE figures. Out of thirty-seven examined cases, twenty-nine showed values below the average, and twenty-one showed values below the lowest range of total cholesterol in the respective age groups.

In Graphs III and IV (p 564), the total cholesterol and the ester ratio in fifteen cases of B T malaria and twenty-two cases of M T are compared with the results of the Weltmann Reaction. Although the majority of malaria patients show a definite decrease in the ester ratio, there is no correlation between the rate of this decrease and the shift to the right of the Weltmann Reaction. A comparison of the results of the red blood cell counts, haemolysis determination, W R and cholesterol partition (shown in Table VII) indicate that there is also no correlation between the rate of decrease of the ester ratio and anaemia or the sensitivity of the blood to lysis in bile solution.

No clear correlation between the total cholesterol values, anaemia and haemolysis, could be found. NOCHT was the first to draw attention to the correlation of hypercholesterinaemia and haemolytic symptoms in blackwater fever. He and his co-workers found that rabbits whose sera had been enriched with cholesterol, tended to be less sensitive to haemolytic substances. GRIMM, OTT, MATTHIEU, DE RAYMOND and COSTILLON even used cholesterol as therapeutic means to check haemolysis in man. (Cited by NOCHT and MAYER, 1937). FAIRLEY and BROMFIELD reported very low values in their cases of blackwater fever. A causative relationship has not however been proved, and NOCHT and MAYER (1937) state that they have not always been able to establish a clear connection between haemolysis in malaria and a cholesterol deficiency in the blood.

TABLE VII

Ester ratio.	Total number of cases.	Weltmann reaction					Haemolysis in hours.					R B C. in millions below							
		6	7	8	9	10	3	2.5	2	1.5	1	2.5	2	1.5	1	4	3.5	3	2.5
1 0-1	1 0-5	5	1	1	1				1	2	1			1			2		1
1 0.6-1	1	10	1		7	2	2	2		2	1					1	4	2	1
1 1.1-1	1 1.5	14	1	2	4	1	2	1	2			1	1			2	5	1	1
1 1.6-1		7	1		2	2	2	1	1		1					1		4	1

A group of nine patients was tested by the Cephalin Flocculation, the Cholesterol Partition Method and the Weltmann Reaction. Pathological results were obtained in seven out of nine patients in these tests. It appears that there exists no correlation between the decrease in the ester ratio and the intensity of the Cephalin Flocculation, one case with 1 0.3 Cholesterol Partition even being negative in the Cephalin Flocculation Test. This is not very surprising, however considering that even in marked impairment of some function of the liver others may be undisturbed. Thus DROG, in discussion of the study of hepatic function in therapeutic malaria by FARMER and HORBAUER (1945), mentions that they did not observe significant changes in prothrombin concentration in a group of fifty nine patients tested weekly.

A value of 6 or 7 in the Weltmann Reaction signifies normality only in healthy individuals. This statement from WELTMANN and the pathological results of the Cephalin Flocculation, Cholesterol Partition and haemolysis in patients showing Weltmann 6 and 7 seems to justify the interpretation of such data in malaria, at least in a certain proportion of the cases, in the sense of a shift to the right.

Instructive in this connection is a comparison of the results of the Weltmann

Reaction with another globulin-flocculation procedure, based on the test introduced by CHORINE. The results obtained with both tests in seventy-one cases are given below

		O	±	+	++	+++	++++
7 cases with Weltmann	6	6		1			
40	" 7	27		4	7	2	
24	8	4		4	6	9	1

Dilution of serum in distilled water, 1 30

The Weltmann Reaction reflects alterations of the serum globulin, the formation of which takes place mainly in the liver. The Weltmann figures indicate, therefore, the state of this liver function. This does not mean that the WR is a more reliable liver function test. In fact, there are no true liver function tests, only specific singular functional tests (ALLEN). On the other hand, it is doubtful whether it is justified to declare Cephalin-cholesterol Flocculation more dependable than other tests used to determine globulin alterations, giving positive reactions in a similar proportion of cases.

Haemolysis as the only cause of abnormal results in the Weltmann Reaction cannot be accepted. It is easy to produce *in vitro* a shift to the right by artificial haemolysis. There is some ground for assuming that rapid haemolysis will shift the WR to the right, but it remains unexplained why in malaria these changes, if due only to haemolysis, remain unaltered during anti-malarial treatment which has been found to enhance sensitivity to lysis. We should have expected a further shift to the right to follow during treatment with quinine, but this does not occur. The persistence of the Weltmann figures, in periods of decreased sensitivity to lysis, 2 to 4 weeks after treatment, indicates, too, that haemolysis is certainly not the only factor.

The second reason which would explain the shift of the coagulation band is toxic liver damage, conclusively demonstrated in numerous studies on hepatic disturbances in induced and naturally acquired malaria.

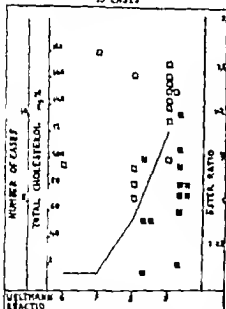
Chronic proliferative processes can, of course, in acute primary infections, be excluded as the factor influencing the shift to the right which appears even after shortest duration of illness.

Comparison of the Weltmann Reaction with other generally accepted liver function tests proves again the limitations of function tests in general, especially if only one determination is made. The results of tests of the various functions of the liver only render it possible to estimate more accurately the extent of existing damage. Repeated determinations during the course of the disease are obviously of far greater diagnostic and prognostic value than one single

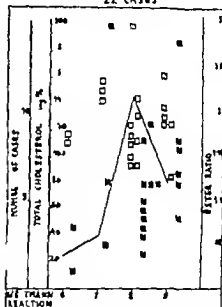
determination. The unchanged W.R. before and after treatment suggests that treatment of malaria by quinine or mepracrine does not involve serious danger to the liver.

We are not inclined to conclude that protective measures for the liver have to be taken in every single case of malaria, but rather to stress the importance of early diagnosis and prompt treatment of the infection, a procedure that seems to be of value in the prevention of disturbances of the liver function.

GRAPH III  
P. VIVAX MALARIA  
15 CASES



GRAPH IV  
P. FALCIPARUM MALARIA  
22 CASES



□ TOTAL CHOLESTEROL    ■ ESTER RATIO    — WETMANN REACTION

This is seen from the results of tests in our group of relapsing B.T. malaria and is confirmed by Korr and Solomon (1943) for induced malaria, as well as by Lippincott *et al.* (1945) in their large-scale experiments on chronic relapsing B.T. malaria. Under unfavourable conditions, such as under nourishment, hard labour chronic intestinal or liver disease, however an acute malaria infection may exert a heavy strain on the liver. A special protective liver treatment, besides efficient specific chemotherapy will under these conditions be of great value.

There are many tropical and cosmopolitan diseases which may clinically be mistaken for malaria. Blood examination will, of course, in most cases





Proliferative changes can be excluded as a cause influencing the W R. because even marked shift to the right occurred after the shortest duration of an acute infection.

Haemolysis most certainly plays a role in the mechanism of the W R. in acute malaria.

Toxic liver damage is evident in results of all applied function tests. The W R. and the Chrome Test, positive in a significant number of acute malaria cases, are based on certain serum globulins. The production of globulins takes place mainly in the liver and therefore globulin alterations are indicative of liver damage.

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## SPLENECTOMY IN THE TROPICS

By

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In the tropics splenectomy is frequently a formidable operation. The massive spleen, the resultant displacement of organs and the dense enveloping adhesions render safe removal difficult. Furthermore, the lowered vitality of most patients demands careful pre-operative assessment, the incidence of surgical shock, both during and after operation, being high. Blood and plasma transfusions have appreciably reduced risk in the profoundly anaemic.

Surprisingly little trauma can rupture a congested malarial spleen, yet accident cases comprise only a minority of splenectomies undertaken in the tropics. The majority are performed on people reduced to chronic ill health by various tropical affections implicating the spleen. The incessant pain and discomfort of an enlarged spleen, dragging on attachments and encroaching on neighbouring organs, compel them to seek treatment.

Occasionally large spleens are present in young subjects with hepatic cirrhosis, but whether such association bears any causal relationship one with the other, as in Banti's disease, is difficult to determine for hepatic cirrhosis even in young people is prevalent among the poorly fed classes.

The sickle-cell trait in negroes, characterized by haemolytic manifestations, is sometimes attended by splenic enlargement. In one such case, after splenectomy, sickling of the red cells was demonstrated in the splenic pulp, together with areas of tuberculous caseation, subcapsularly situated.

Miscarriages are frequent through mechanical arrest of the gravid uterus

Common causes of tropical splenomegaly are malaria, schistosomiasis and leishmaniasis. The last named is rarely encountered in West Africa.

Before removing spleens in which malaria has contributed to enlargement, a course of anti malarial therapy often reduces size appreciably. During treatment a retracting spleen evokes characteristic aggravation of pain which, in fact, constitutes a subjective sign of therapeutic response.

Spinal anaesthesia is satisfactory in practically all cases, but, where caution in exceptionally poor surgical risks prohibits its use at the necessary high level, supplementary cyclonal sodium maintains adequate relaxation.

In order to gain comfortable access to a large spleen, it is necessary sometimes, in addition to a paramedian or rectus splitting incision, to make an upper transectus incision extending well beyond the lateral border of the muscle. To minimize shock when freeing the spleen a half per cent. novocaine is streamed around its deeper attachments.

Usually the first important intra-abdominal procedure in freeing a non-adherent spleen is to trace and identify the pedicle, whose ligation and division is facilitated by raising the organ from its bed. The slack of both gastro-splenic and lienorenal ligaments ensures sufficient mobility for this manoeuvre. Because tropical spleens are usually so adherent at their convexities with the costo-diaphragmatic peritoneum this manipulation is impossible until adhesions here are separated.

Great care is necessary in dividing attachments with neighbouring organs. The tail of the pancreas and greater curvature of the stomach are usually densely enveloped within compressed omental folds, which are all firmly adherent to the abutting spleen.

When stomach, pancreas and adjoining coils of gut are sufficiently freed, the deeper adhesions in the splenic bed remain to be dealt with. Not until the diaphragmatic surface of the spleen has been rendered completely mobile by thorough separation of adhesions should any attempt be made to divide in whole the tortuous vessels of the pedicle. Accessible vessels can, of course, be safely ligated one by one, but determined search for the whole pedicle is too hazardous at this stage, and should be deferred while the spleen remains fixed in the depth of its bed. Only after the spleen has been rendered completely mobile can the whole pedicle be identified and safely ligated.

Rapid and effectual separation of the adherent spleen from its bed requires considerable resolution, but surprisingly little bleeding ever arises from the denuded areas. Only pinpoint haemorrhages occur which are promptly controlled by hot packs before a general ooze sets in.

Separation is best carried out by thrusting the hand behind the convexity and boldly breaking down all adhesions digitally wherever encountered. This phase of the operation probably produces most shock.

When all adhesions are freed, the spleen is carefully raised from its bed, a watchful eye being kept on the large and sometimes friable pedicle, whose identification and division now become easy.

Once satisfied that oozing from the splenic bed has ceased, and that divided vessels are securely ligated, the abdomen can safely be closed without drainage. The following case histories exemplify most of these points.

### CASE 1

Splenomegaly in an African tin-mining labourer, aged 26, associated with the sickle-cell trait, ankylostomiasis and urinary schistosomiasis.

*History*—Nine months pleuritic like pain, general weakness, dyspnoea, with oedema of face and legs. The spleen was palpable a hand's breadth below the left costal margin. Cystoscopy revealed no gross bilharzial lesions.

Helminthiasis was appropriately treated, and after ten injections of emetine the urine was free of terminal spined ova and R B C s.

The erythrocyte count just before operation rose to 4,920,000 Hb 80 per cent, C I, 1.08.

Mepacrine and quinine caused no appreciable retraction of the spleen.

*Anaesthesia*—Pre-operative morphine sedation, followed by 12 c c spinal percaïne, supplemented at intervals during operation with intravenous cyclonal sodium totalling 10 c c.

*Operation*—Right paramedian and upper transrectus incisions. The enlarged spleen was firmly adherent to the costal parietes, particularly at the upper pole, where there was dense white scarring of the capsule. Adhesions were digitally freed, and the spleen raised from its bed. A large pedicle was clamped segmentally, and ligated close to the hilar surface. Recovery was uneventful.

*Pathology of Spleen*—Capsular thickening, marked congestion of pulp with sickling of R B C. Moderate fibrosis of the walls of the splenic sinuses. The malpighian bodies were on the whole well preserved, though the central arteries showed degenerative changes, and, in some, the lymphoid tissue also showed early degeneration. The sickle-cell trait was also present, but whether the other changes were due to sickle-cell disease it was difficult to say.

### CASE 2

Chronic splenomegaly in an African house boy, aged 27.

*History*—Persistent pain and a feeling of heaviness in the left side aggravated when running or during any strenuous physical exertion. Since childhood he had always been aware of a lump in the left side.

Examination disclosed a large multiple notched spleen markedly tender on pressure.

Blood slides were negative for malarial parasites, and there were no bilharzia ova in the urine.

The erythrocyte count at operation was 4,460,000 Hb, 65 per cent, C I, 1.07.

*Anaesthesia*—Pre-operative morphine sedation was followed by spinal percarne 12 c.c., supplemented during operation with cyclonal sodium.

*Operation*—Left rectus muscle splitting and upper transrectus incisions. The spleen presented easily as there were no firm costal adhesions. The large vascular pedicle containing a tortuous splenic vein was quickly identified, clamped and ligated. A massive quadrupled notched spleen was removed.

The patient was discharged 3 weeks after operation in excellent health.

*Pathology of Spleen*—Both the gross appearance and histopathology were characteristic of chronic malaria.

### CASE 3.

Chronic splenomegaly in an African tin-mining labourer aged 25

*History*—A painful splenic swelling of several years' duration, which in the few days before admission to hospital had rapidly enlarged with increasing pain.

*Examination*.—A large tense, painful and extremely tender spleen was palpated. The patient was afebrile. The erythrocyte count was under 3 000 000. Hb. 55 per cent., and a wet blood preparation showed much sickling. A white cell count of 9,200 showed polymorphs 38 per cent., lymphocytes 43 per cent., monocytes 1 per cent., eosinophils 18 per cent. No malaria parasites were found in the peripheral blood.

*Anaesthesia*—Pre-operative morphine sedation was followed by spinal percarne 12 c.c. supplemented during operation with interval doses of cyclonal sodium to a total of 10 c.c.

*Operation*.—A left paramedian rectus splitting incision. Portions of omentum were adherent to the parietal peritoneum and to the anterior surface of the spleen. The transverse colon was tacked to the lower pole. After separating dense costo-diaphragmatic adhesions digitally a heavy roughly quadrilateral shaped spleen was lifted from its bed where oozing was easily controlled. The pedicle was clamped and doubly ligated. In handling the spleen white creamy pus exuded at various points through the tense capsule.

Recovery was uneventful, and he was discharged from hospital a month later.

*Pathology of Spleen*.—There was diffuse fibrosis of the pulp with atrophy of the malpighian bodies, some haemorrhages and well-marked sickling of R.B.C. A caseating tuberculous area was also present.

### CASE 4

Chronic splenomegaly in an African male, aged 17

*History*—Failing health and increasing left sided pain of several months duration.

*Examination*—A tall emaciated youth weighing only 68 lb. with a large tender spleen. He had a purulent conjunctivitis and symptoms of acute

bacillary dysentery Both were treated Flagellates were present in the stool The urine contained casts and albumin, but no ova

The erythrocyte count on admission was 2,610,000 Hb, 55 per cent, C I, I O Owing to his extreme impatience after admission to hospital, and his threat to leave at once unless operated on immediately, splenectomy was undertaken only 5 days after admission.

*Anaesthesia*—Morphine sedation was followed by spinal percarine 14 c c

*Operation*—Left paramedian and bilateral transrectus incisions were necessary because of the great size of the spleen, which was firmly adherent to the costo-diaphragmatic peritoneum Adhesions here were separated digitally and the lower pole raised The pedicle was clamped segmentally Glucose and saline infusions were administered intravenously and rectally on return to the ward

Recovery was uneventful He failed to report in 6 weeks' time, as requested, but was last seen 3 months later working on a tin-mine, having gained considerable weight

*Pathology of Spleen*—Except for the markedly thickened and hyaline capsule and diffuse thickening of the pulp, there were no special pathological changes

## CASE 5

Splenectomy for chronic malarial splenomegaly in a married African woman, aged 28, having a history of frequent miscarriages

Pre-operative mepacrine therapy caused only slight, though definite, splenic retraction. A 35 per cent Hb level rose 14 days later to 55 per cent on neo-hepatex injections and fersolate tablets, and at this stage operation was undertaken

*Anaesthesia*—Morphine sedation, followed by 13 c c nupercaine, supplemented during operation with cyclonal sodium in repeated interval dosage of 3 c c

*Operation*—The abdomen was opened and access gained to the spleen by the incisions previously described The anterior surface of the enlarged spleen was wholly covered by adhered omentum, whose division revealed the greater curvature of the stomach beneath, closely adherent to the concave hilar surface of the protruding spleen The compressed gastro-splenic ligament obscured the tail of the pancreas In spite of careful clamping of the pedicle close to the hilum, a segment of the obscured greater curvature was caught up and incised with it, thus opening the stomach The edges of the rent were carefully sutured in three layers

Nine days later the abdominal wound bulged slightly and gave a crepitant sensation She coughed and omentum presented through a gap The edges were approximated with through and through silkworm sutures in figure of eight

fashion, and sulphonamide powder sprinkled on the wound. Healing was sound and her recovery was excellent.

### CASE 6.

Splenectomy following traumatic rupture in an African soldier aged 28.

This soldier fell from a military lorry sustaining small abrasions over both knees and what appeared at the time to be slight bruising on the left loin. He walked for  $\frac{1}{2}$  mile unaided to the African hospital, and on admission was seen by an A.M.O. The pulse was 68 temperature 97° F. "Slight resistance over epigastrium tenderness over left upper abdomen in splenic area. Seen later the same evening he was comfortable. The pulse had not risen and, recorded hourly throughout the night, the rat varied between 78 and 90 till next morning when he was still restful, having slept well. At 10 a.m., however his condition suddenly changed after drinking about half a pint of maize gruel. He became distressed, respirations were hurried, the abdomen rapidly distended, and his features assumed a dehydrated appearance. He was given morphine and brought to the theatre where, owing to the absence through illness of the European theatre sister responsibility devolved on the African theatre staff. With 2 per cent. plaincaine in saline, a block anaesthesia of the anterior abdominal wall was rapidly done. A left paramedian rectus splitting incision exposed the posterior sheath, when intravenous cycloal sodium was given. The abdominal contents were under great pressure, and on incising the peritoneum a considerable quantity of blood escaped. The remaining blood in the abdominal cavity was soaked up in large gauze packs which, after citration in a sterile bowl, was auto-transfused through a 50 c.c. three way syringe while the operation proceeded. In this way he received back 2 pints. Access to the spleen was rendered easier by the usual upper transrectus incision. The large spleen had few parietal adhesions and was easily removed. An inch-wide strand of omentum adhered to its lower pole. The pedicle was partially torn through. A deep gaping tear filled with dark clot traversed the lower third of its convexity to a depth of 3 inches. The torn pedicle was divided and securely ligated. Rectal saline was administered before return to the ward.

Later in the day owing to haecorrh, he was placed in the semi-recumbent position, which greatly eased him.

Four days later the erythrocyte count was 2,500,000 Hb., 55 per cent. C.I., 1.0. The urine, containing *Schistosoma haematodum* ova and R.B.C.s, was treated over a period of 12 days and cleared by emetine 3 grains and anthiomaline 25 c.c. Except for some irregularity of temperature for several days, convalescence was uninterrupted.

It is clear that the quiescent phase following on the actual splenic rupture ended by his taking a meal next morning. The consequent gastric peristalsis and the drag of a partly filled stomach dislodged clots which for some hours had sealed the splenic tear.

## CASE 7

Splenectomy 3 days after traumatic rupture of the spleen in an African boy aged 7 years

The boy was brought to hospital at 7 p m by his mother. No mention was then made of the slight fall he had sustained on his way from school 3 days previously. There was no external bruising nor had noticeable symptoms occurred until an hour or so before admission, when he suddenly became distressed. The abdomen was distended. Generalized tenderness and rigidity had set in. Auscultation—a silent abdomen. Temperature, 100.8° F. Pulse, 104. Bowel and bladder were functioning normally.

Some days later an observant relative disclosed that the child had complained of pain in the left flank, aggravated by raising the left arm, and by bearing his weight on the left foot.

In the absence of a satisfactory history, exploratory laparotomy was done 2 hours after admission.

*Anaesthesia*—Intrathecal percarne 8 c c, supplemented during operation with cyclonal sodium 5 c c and 4 c c (No morphine premedication.)

*Operation*—Right infra-umbilical paramedian incision. Appendix normal. Intestinal coils were collapsed and streaked with blood. No early bowel perforation. The pelvis contained a quantity of blood. Incision was extended upwards and a mobile, slightly enlarged spleen palpated. Its convex surface was free from adhesions and here the fingers sank into a boggy, ragged-edged depression. A transrectus incision brought the whole organ into view. The hilar surface was obscured by blood clots and by impinging rolled-up omentum. The pedicle, not easily identifiable, was clamped and doubly ligated, being first put on the stretch by raising the spleen vertically. When removed, the smooth convex surface showed deep stellate fissuring. The hilar surface was also fragmented. Except for slight enlargement, there was nothing else notable in the pathology of the spleen. Convalescence and recovery were uneventful.

This and the preceding case well illustrate the quiescent symptomless phase that sometimes follows splenic rupture.

## SUMMARY

The usual causes of tropical splenomegaly in West Africa are mentioned. Splenectomy as a means of relieving pain and discomfort, and improving general health in certain chronic cases, is discussed. Pronounced sickling of the red cells in two instances suggests a possible contributory rôle by this trait in splenic enlargement among negroes. Seven typical case histories serve to illustrate points in operative procedure. The safety of intrathecal analgesia in sub-diaphragmatic operations of this nature is worthy of note.



## CORRESPONDENCE.

## THE AETIOLOGY OF DESERT SORE.

To the Editor *Transactions of the Royal Society of Tropical Medicine and Hygiene*

In criticizing Colonel S. T. ANNING a paper on the aetiology of desert sore the discussion of small sample technique was deliberately avoided,† although it was indeed felt that his numbers were on the small side. However since in Colonel ANNING's reply‡ he uses the smallness of his series to justify the technique adopted it must in the interests of accuracy be pointed out that in fact this technique offers no advantage in that respect over the one which he states was discarded.

In fact the only really appropriate test of significance in such relatively small series as that under consideration appears to be Student's *t*-test. The application of this is not at all difficult and it is perhaps unfortunate that BRADFORD HILL's *Medical Statistics* which occupies such a unique position in statistical literature, does not find room for it. However a most readable and lucid discussion will be found in *The Treatment of Clinical and Laboratory Data* by Professor DONALD MAITLAND (Oliver and Boyd, 1938), a book which is warmly recommended to anyone who has idea of publishing the results of small-scale researches of any description.

Colonial Medical Service  
Nigeria.

I am, etc.,

J. L. LESTER M.D.

ANNING, S. T. (1946) The aetiology of desert sore. *Trans. R. Soc. trop. Med. Hyg.* 40 (3) 313.

† LESTER, J. I. (1947) Correspondence. *Ibid.* 41 (5) 909.

‡ ANNING, S. T. (1947) Correspondence. *Ibid.* 41 (2) 208.

## CORRIGENDA

GONZALEZ, L. M. *et al.* (1947) Studies on the genus *Shigella*. 41 (1) 93.  
Page 99 (9th line from the bottom of the page, and also 1st line)

for (See Chart 1) read (See Chart 2)

Page 100 Chart 2.

Between the upper and lower halves of the chart insert Type II (W) as heading to the lower half

Page 101 Chart 3

Between the upper and lower halves of the chart insert Type I III (V7).

13th line from the bottom of the page for (See Chart 2) read (See Chart 3)

Page 102 (6th line).

for (See Chart 3) read (See Chart 4)

PANDEY, R. (1947) Mixed deficiency diseases in India. 41 (2) 189.  
Page 201 (5th line from bottom of page).

for vitamin A (aneurin) therapy read vitamin A therapy

Page 201 (4th line from bottom of page)

for Vitamin B<sub>1</sub> (thiamin) read Vitamin B (thiamin or aneurin)

Page 203 (heading of second paragraph).

for Treatment of Carcinoma Infections read Treatment of Concurrent Infections.

Vol. 41 \ 3 Dates in box heading at top of page to be corrected as below  
Page 265 for 1948 read 1947 Page 377 for 1948 read 1947 Page 419 for 1938 read 1917

## ANNOUNCEMENTS.

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### NEXT MEETING OF THE SOCIETY

The next meeting of the Society will be held at Manson House, 26, Portland Place, London, at 7 30 p m on Thursday, 19th February, 1948. Paper on "Pathological processes in malaria," by Professor B G MAEGRAITH and Dr W H H ANDREWS of the Liverpool School of Tropical Medicine

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### MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are temporarily in the British Isles. Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address.

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad.

AL-ABED, H E, Iraq  
 ALEXANDER, G L, Gold Coast  
 CAFFEY, P J, Nigeria  
 CHARTRES, J C, Nigeria  
 CLARK, MILDRED, Natal  
 CLARK, R H P, India.  
 CUMMINS, Lt -Col P H, India  
 DAVIDSON, Lt -Col T J, India  
 DAVIES, C W, Kenya  
 DAVIS, D H S, South Africa  
 DICK, G W R, Uganda  
 DIXON, P, Belgian Congo  
 DUGGAN, A J, Nigeria  
 EGWUATU, O S, Nigeria  
 FIELD, J W, Malaya  
 FOY, HENRY, Greece  
 GRAHAM-CUMMING, G, Hongkong  
 GREANY, W H, Sudan  
 GREENING, Major C LL, India  
 HARKNESS, J W P, Nigeria  
 HUNTER, W, Nigeria.  
 IP, YEE Hongkong  
 KENT, Lt -Col P W, India  
 KHAYATT, SAMI, Iraq  
 LINDSAY, Lt -Col D K LL, India

LING, D T H, Hongkong  
 MCLEISH, A C, India  
 MAGUIRE, E H C, India  
 MORTON, T A, Gold Coast  
 PAL, RAJINDAR, India  
 PHILLIPS, C M, N Rhodesia  
 QUANTRILL, D W, Nigeria  
 RITCHIE, G L, Tanganyika  
 RUSSELL, S F, Assam  
 SAUNDERS, G F T, Gold Coast  
 SCRIMGEOUR, H, Singapore  
 SMITH, CONSTANCE B, Malaya  
 SMITH, Lt -Col M C L, India  
 TO, S SHIU-YUEN, Hongkong  
 UTTLEY, K H, Hongkong  
 WATERMAN, J, Trinidad  
 WATT, Lt -Col GEORGE, Gold Coast  
 WHITE, T H, Tanganyika  
 WIGAN, W C, Nyasaland  
 WILKINS, E G, India  
 WILLIAMS, W R, Gold Coast  
 WILSON, C J, Kenya.  
 WILSON, W A, Uganda  
 WING, W, M, U.S.A.

### NEW FELLOWS

At the meeting of the Society held at Mansion House on 11th December 1947 the following nine candidates were elected Fellows of the Society —

ALBERT ADRIEN B.Sc. (STD.), PH.D. MEDICINE (LOND.), F.R.I.C., London.  
 D'ANTONI, JOSEPH S. M.D. Professor of Clinical Tropical Medicine U.S.A.  
 GOW, KOK AUN M.B. B.S. (HONGKONG) Hongkong.  
 KENT ROGER P., M.B., B.S. (LOND.) London.  
 SLOAN THOMAS B.M. M.B. CH.B. (EDIN.) Scotland.  
 SMITH, MICHAEL H., B.Sc. (LOND.), Nigeria.  
 TSIUNG, FAT IM, M.B., B.S. (HONGKONG) Hongkong.  
 VILEN ARTHUR F. M.D. (STOCKHOLM) Paediatrician, Belgian Congo.  
 WING, WILSON M., A.B. (HARVARD), M.A. (CANTAB.) M.D. (COLUMBIA), M.P.H. (JOHNS HOPKINS) U.S.A.

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At the meeting of the Society held at Mansion House on 15th January 1948 the following eight candidates were elected Fellow of the Society —

ARKELL, E. D. M.B., B.S. (LOND.) M.R.C.S., L.R.C.P. Swaziland.  
 CHOO TAN HEE, M.B., B.S. (HONGKONG), Hongkong.  
 DAVIS DAVID H. S. M.A. (OTUM), South Africa.  
 DICKIE, ROBERT M.B., CH.B. (GLAS.), NETHER.  
 GONZALEZ, LUIS M., M.B., PH.D. (PISCO), West Indies.  
 HAMILTON, WALTER HOOES, M.B., B.S. (LOND.), M.R.C.S., L.R.C.P. Egypt.  
 LEE, ROBERT PEARSON, M.R.C.V.S. (DUBLIN), England.  
 WONG, YAT HUNG, M.B., B.S. (HONGKONG) B.Sc. (SHANGHAI), China.

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### ADMISSION TO FELLOWSHIP OF THE SOCIETY

All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society.

Anyone desiring to become a candidate for Fellowship of the Society should use the special form of application at the end of this journal.

The annual subscription payable by Fellows is one and half guineas (£1 11s. 6d) which becomes due in advance on the 1st of April of each year.

The TRANSACTIONS and the current YEAR BOOK of the Society are posted regularly to every Fellow whose subscription is not in arrear.

Further information may be obtained from the Hon. Secretaries, Mansion House 26, Portland Place, London, W 1; or from the Local Secretary of the district.

## NOTICE TO FELLOWS

As a copy of each number of the TRANSACTIONS is posted to every Fellow whose subscription is not in arrear, Fellows are particularly requested to notify the Secretaries of any change in the address to which their TRANSACTIONS are to be posted

When copies of the TRANSACTIONS are returned by the Post Office marked "Gone Away," "No Service," or "Insufficient Address," no more copies will be posted to that address but they will be retained at Manson House until further instructions are received

## MEDALS AND PRIZES TO BE AWARDED IN 1948

### CHALMERS MEDAL

At the Council held on 10th April, 1947, it was decided to award in 1948 the three outstanding CHALMERS MEDALS, namely, those due in 1941, 1943 and 1945 respectively

Any Fellow is entitled to make a nomination. The proposer shall deposit with the Honorary Secretaries of the Society at Manson House, not later than 23rd FEBRUARY, 1948, a statement which must include the following data

- (a) Date of nominee's birth
- (b) A detailed statement of nominee's claim for consideration, indicating the year or years in which any work mentioned was carried out
- (c) A list of the nominee's published papers with dates

Full particulars are given in the YEAR BOOK of the Royal Society of Tropical Medicine and Hygiene

### THE CONSULTANTS PRIZE

The Consultants to the War Office and the Armies in the Field in the late war, have presented a sum of money to the R A M C in order to found a Consultants Prize, to be competed for at intervals of 1 to 3 years

This prize will be awarded for the first time in 1948 and will be to the value of 25 guineas. The prize is open to serving officers of the Royal Army Medical Corps, holding a regular or a short-service commission

The first prize will be awarded for an essay of not more than 10,000 words on a professional subject, based on the author's own experiences between 1939 and 1946. It is hoped that these essays will ensure that valuable war experience which would otherwise be lost, will be recorded for future guidance and possibly for publication

Entries should be sent in through the usual channels, so as to reach the Hon Secretary, R A M C Prize Funds Committee, R.A.M. College, Millbank, London, S W 1 not later than 1st AUGUST, 1948

## LIBRARY NOTICES

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Of the *Chinese Medical Journal*, Volumes 62 & 63 (1944-1945) are incomplete.

Of the *Journal of Tropical Medicine & Hygiene* No. 4 of Volume 49 (1947) is missing.

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*Conferência Intercolonial sobre Tripanossomíases*. Vol. 1. Lourenço Marques: Imprensa Nacional de Moçambique.

*Índice Bibliográfico de Lepre*. Vols I and II by L. KERRA. São Paulo: Library of the Leprosy Prevention Department.

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<i>Arch. Schiffs u. Tropenhyg.</i>	<i>Dtsch. med. Woch.</i>	<i>Trans. R. Soc. trop. Med. Hyg.</i>
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centimetre cm.	micron, $\mu$ .	ounce, oz.
cubic centimetre, c.	milligramme, mg.	pound, lb.
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# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

MARCH, 1948

VOLUME 41

No 5

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VOL 41 No 5 MARCH, 1948

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ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,  
on

Thursday, 15th January, 1948, at 7 30 p m

THE PRESIDENT,

SIR PHILIP MANSON-BAHR, C M G , D S O , M D , F R C P ,  
in the Chair

---

SYMPOSIUM ON LEPROSY.

---

PAPER

---

RECENT ADVANCES IN THE TREATMENT OF LEPROSY

BY

ERNEST MUIR, C I E , M D , F R C S E

---

During the last 30 years there have been scores of remedies put forward as of use in the treatment of leprosy, generally by doctors with little or no experience of the vagaries of the disease

Leprosy, at least in its severer forms, advances slowly and insidiously Like benign tertian malaria it has its acute attacks, but in neither disease does the subsidence of an acute attack mean that the patient is any nearer recovery Too often the striking clinical improvement accompanying this subsidence in leprosy has been credited to some drug which happened to have been given at the time One has therefore to be very careful in estimating the effects of any drug in leprosy

*Hydnocarpus Oil* Personally, although I have used hydnocarpus oil and its preparations in treating leprosy for the last 30 years, I am not entirely persuaded that it has much special effect in leprosy apart from its local counter-irritant action on skin lesions when given intradermally, and possibly a general

counter-irritant effect when given intramuscularly I know that such a view is regarded as heretical by some of my experienced colleagues. There is little doubt that leprosy varies from place to place in its severity and in its amenability to hydnocarpus treatment. In Calcutta I used to get far better results than I did in Trinidad and perhaps it is my experience in the latter place that has made me pessimistic of the effects of hydnocarpus oil.

One of the great drawbacks in leprosy is the impracticability of carrying out a properly controlled therapeutic experiment. I have tried it often and always failed, nor do I know of anyone who has succeeded any better.

For such an experiment it would be necessary to eliminate all subsidiary factors and to set case against absolutely comparable case, the only difference being that one is given the drug to be tested and the other without knowing it, is given something quite different. But however alike each other the two cases may be to begin with, long before the prolonged period needed to get results has passed, the comparability of the two cases will have been changed by intercurrent causes that have no connection with the experiment and thus the results are vitiated.

#### SULPHONES.

As compared with the doubtful opinions about hydnocarpus oil there is a wide consensus of opinion that we have now in the sulphones a remedy which is definitely beneficial. Whether or not it will be possible to test out their efficacy in a controlled experiment, as has been proposed in America I am doubtful. If this were to be done it would probably be necessary to confine the controls in a separate institution without any communication with those under treatment for it is scarcely compatible with human nature for Group A of sufferers from leprosy to be in contact with Group B and that the latter should be content to watch the former getting gradually better through months and years, while they themselves are allowed to get steadily worse.

As far as I can judge, the true criterion of the efficacy of the sulphones must be based not on the usual form of controlled experiment, but on the judgment of experienced specialists who are capable of making fairly accurate prognosis in typical cases of leprosy. A patient in whom the disease has been progressing steadily for years begins to improve within a few weeks of beginning the treatment. His ulcers of months, or years, duration quickly heal. His nose, long blocked with crusts becomes clear. His eyes deteriorating rapidly towards complete blindness, have the disease—a shown by the slit lamp and corneal microscope—quickly arrested. All these give reason for hope but it is when after 1 to 3 years treatment the massive infection in the skin and nose gives place to a condition in which bacilli cannot be found, or are found only with difficulty that the true value of the sulphones can be appreciated.

This is a picture which I have found in not one only but in almost all

the cases that have been under treatment for a sufficient period of time I consider that if equally good results are obtained throughout the world (and reports received indicate that this may be so) then no further controls are necessary in proving that treatment with sulphones is at least a very definite advance

#### ORIGIN OF SULPHONE TREATMENT

The credit for the first use of sulphones in leprosy is due to the workers at the National Leprosarium, Carville, Louisiana (FAGET *et al*, 1943, 1946) particularly to the late Dr G H FAGET (1947) The effects of promin in guinea-pigs (FELDMAN, 1942) in controlling tuberculosis led the Carville workers to use this drug in leprosy They found it too toxic to give by the mouth, but given intravenously it was tolerated in doses up to 5 grammes a day

#### CASES TREATED WITH PROMIN

The majority of the patients at Carville are advanced cases, and the following results have recently been reported of thirty-eight patients under treatment for 2 years, the disease is arrested in three, of eighteen treated for 3 years, arrested in three cases, of forty one, for 4 years, arrested in six, of eight, for 5 years, arrested in seven cases Thus it would appear that at least in fairly advanced cases promin takes about 5 years to cause arrest of the disease in the majority

#### CASES TREATED WITH DIASONE

In 1944, when in charge of the leprosarium in Trinidad, I read the preliminary report of the Carville workers, and I tried at once to get a supply of promin, but failed at first to do so The Abbott Laboratories, however, kindly sent me a supply of diasone and I was able to get a number of patients under treatment by the middle of 1944 In all my experience of leprosy I had never seen such bad cases of the disease as there were in the Trinidad leprosarium I therefore chose, in addition to earlier cases, some of the more serious ones patients who were becoming blind, who were having great trouble with the blocking and crusting up of the nose, and were suffering from large lepromatous ulcers which would not heal I was at first afraid that, like promin, diasone might be toxic if given by the mouth, and therefore gave it intravenously Later I found that it was well-tolerated by the mouth

The results in the first cases treated were remarkable within the first few weeks (MUIR, 1944) the disease subsided in the eyes, and vision improved, noses became clear and ulcers healed rapidly Patients who had been bed-ridden for months with fever were able to get up and work I was not, however, at all sure whether these improvements were due to any specific action on lepra bacilli or simply to the effect on complicating septic organisms The patients themselves were very much impressed and I was besieged with requests from others to be put on to the treatment roll

Of one danger I very soon became aware A patient who had long been

crippled with ulcers and suffered from fever had recovered quickly and engaged in work. After a few weeks he became rapidly weak and short of breath and I found that he was suffering from acute anaemia, with a haemoglobin percentage of 25. With large doses of iron by mouth and liver injections he soon recovered and was able after a few weeks, to continue the diasone treatment. As I left Trinidad in February 1945 I was not able to form any definite observation on the diminution of the number of lepra bacilli.

On returning to this country I was able, through the kindness of Abbott Laboratories, and the courtesy of Dr W. E. COOK and those in charge of the St. Giles Homes to continue trials of diasone in the Homes and elsewhere. Some of these patients have now been on diasone for ½ years and without exception they have all shown satisfactory improvement. Some of them were suffering from chronic lepromatous ulcers and their eye involvement was going on to complete blindness. Colonel KIRWAY will tell you in his paper what was the effect of diasone on their eyes.

In cases in which there was manifest bacillary infection bacilli cannot now be found or are found only with difficulty.

#### *Improvement under Diasone*

In advanced cases of leprosy improvement under diasone treatment may be divided into three stages —

- 1 The cutaneous ulcers heal, the nose becomes clear and the acute progressive condition of the eyes is arrested in a few weeks or months.
2. Febrile reaction and the appearance of transient nodules gradually get less and disappear and nodules become flattened in a period varying from a few months to 1 or 2 years.
- 3 The bacilli become noticeably less until in 2 to 3 years they are hard to find.

#### *PROMIN AND DIASONE TREATMENT IN BRAZIL.*

Sulphones have been used for leprosy much more extensively in Brazil than in any other country. I had an opportunity last year of seeing some of the 400 cases that Dr LAURO DE SOUZA LIMA had under treatment at the Padre Bento Institution, near São Paulo. Half of these were on promin and half on diasone. The patients were photographed, biopsy sections examined, and the lepromin test done at frequent intervals. I was much impressed by the improvement especially in early lepromatous cases. Dr LIMA's opinion, after treating 200 patients with promin for 2 years and an equal number with diasone for 1 year was that of the two drugs diasone gave quicker results, though he did not consider the period long enough to give a final decision. He found that practically every case had improved.

#### *OTHER SULPHONE DERIVATIVES.*

More recently two more sulphone derivatives have been under trial prominazole (2,4-diamino-5-thiazolylphenyl sulphone) and sulpherrone (tetrasodium-

4 4' ( $\gamma$  phenylpropylamino)-diphenyl sulphone  $\alpha$   $\gamma$   $\alpha'$   $\gamma'$ -tetrasulphonate] Promizole (P D & Co) is given orally, and first reports indicate that it is less toxic and quicker in action than promin Sulphetrone (B W & Co) given orally is also proving useful, and early reports indicate that it is less toxic and gives quicker results than the earlier products Diaminodiphenyl sulphone, the nucleus common to all these drugs, has not yet because of its toxicity, been used in leprosy to any great extent It will be interesting to see what effect it has, and whether or not the minimal effective doses are too toxic to be continued over a lengthy period This is in course of being tried out

#### DOSAGE OF SULPHONES

A great deal of work has yet to be done before we know with any certainty how best to regulate the dosage Before treatment, fairly advanced lepromatous cases tend to suffer from anaemia and from reactive exacerbations (lepra reaction) Sulphones tend to increase both these conditions, and even in earlier cases their toxic effects are sometimes seen in causing anaemia and also sometimes fever, along with fugitive nodules and other inflammatory swellings It is necessary therefore to test the blood before beginning treatment and to give full doses of iron if anaemia is present

As far as I am aware, the nature of this anaemia has not yet been fully investigated It has been suggested that sulphones interfere with the absorption of iron from the gut But if that is so it cannot be the only cause, as is shown, for instance by the very rapid production of anaemia in the case mentioned above As in lepromatous leprosy there is frequently a vitamin B deficiency, and this deficiency tends at first to be increased by sulphones, it is well to give full doses of yeast as well as iron

It is well for patients with anaemia, who are subject to reaction, or are in a chronic low febrile state, to begin with small doses, as even small doses are effective in improving the patient's condition and leading him up to a point at which he is gradually able to tolerate larger doses A common beginning in such cases is 0.3 gramme of diasone, or 0.5 gramme of sulphetrone, every second day If this is tolerated without ill effects for a week the dose is doubled, and very gradually increased till the patient is tolerating 1 gramme of diasone, or 2 grammes of sulphetrone on alternate days This point may be reached within a few weeks, or it may take months Thereafter daily doses may be gradually introduced, and then a daily dose of 1 gramme of diasone can be gradually increased to 2 grammes, and 2 grammes of sulphetrone to 3 grammes

In earlier uncomplicated lepromatous cases the dosage may be increased very much more rapidly, as long as frequent examinations weekly or bi-weekly, are made for anaemia While it is well if possible to make white and red cell counts and estimate the colour index, my own experience so far shows that if the initial dosage is carefully regulated on the above lines, the estimation of the haemoglobin percentage is sufficient A fall below 70 per cent is an



indication to stop the drug and press iron giving also if necessary injections of liver preparations. If this simplification of essential tests is confirmed, treatment of large numbers of patients with a limited staff will be very much facilitated.

Febrile reaction and/or the appearance of inflammatory nodes, should be taken as indications temporarily to suspend treatment or diminish the dose the patient being put on alkalis (sodium bicarbonate 1 dram four times daily).

Larger doses than 2 grammes of diarsone or 3 grammes of sulphethione have been recommended by some authorities. It remains yet to be ascertained whether there is sufficient extra benefit in these to justify the extra risk of toxic results. There is reason to believe that sulphonamides concentrate in the skin, mucous membranes and eyes, though I know of no evidence yet as to whether there is high concentration in the peripheral nerves. If it is found that the concentration in these parts is high while giving only moderate doses, and that the concentration is not much increased by larger doses, then, especially in consideration of the long treatment moderate dosage may be preferable from the point of view both of safety and of economy.

#### *Oral compared with Injection Administration*

In many ways it is a great advantage to be able to give oral instead of injection treatment. While the faith of primitive peoples in injections is very high, there are many who get tired of the tyranny of the needle in hydriocarpus treatment and leave off too soon.

There is a danger however in free distribution to leprosy patients in the Colonies, that they may succumb to the temptation to sell tablets in the market instead of taking them themselves or they may try to treat themselves at home perhaps with dangerous results. For this reason possibly an oily suspension of the drug may be found preferable. This has yet to be tried out.

#### MODE OF ACTION OF THE SULPHONAMIDES

We have still no clear evidence as to how the sulphonamides act whether they kill the bacilli or render them incapable of multiplication. The microscopic appearance of acid-fast bacilli in smears and section does not prove whether or not these bacilli are alive. A suspension of nodules containing lepra bacilli may be sterilized by heat and injected into a rat and then numbers of these bacilli, still acid fast, found in the tissues of the rat after 18 months. It may therefore be that many of the acid-fast bacilli found in smears from patients after a year or more of sulphone treatment may be dead. Our inability to culture *Mycobacterium leprae* outside the human body leaves us without conclusive evidence on this point.

Fritz (1946) is of the opinion that sulphonamides destroy the bacilli in the neighbourhood of capillary blood vessels though they persist for a longer period in tissues more remote from blood vessels.

## LENGTH OF TREATMENT

The duration of treatment is another matter on which evidence is required. This may depend to a large extent on whether, as the number of bacilli diminish in the body under sulphone treatment, the patient is rendered resistant to leprosy. This would be indicated by the negative lepromin test, as found in all lepromatous cases, becoming positive. There is some evidence, not yet at all conclusive, that this does occur in a few cases. A report received recently from Dr DAVEY, of the Uzuakoli Leper Settlement in Nigeria, states that of seventeen lepromatous cases under treatment with sulphetrone for 5 to 10 months, the lepromin test has changed to positive in eleven. If this result is confirmed it might not be necessary to continue treatment after repeated examinations over some months had given negative bacteriological results. Otherwise it would be well to continue treatment, perhaps in diminished doses over a still longer period. This matter requires careful and prolonged observation over a period of years before we can have any clear indication.

So far I know of no clear evidence of cases being or becoming sulphone resistant, but it will take a much longer time before it can be said whether or not such cases do occur.

## SULPHONE TREATMENT OF NON LEPROMATOUS CASES

We have no reliable evidence yet as to the effect of sulphones in tuberculoid cases. In the ordinary tuberculoid the bacilli are few and the lesions chronic, the bacilli being comparatively distant from and shut off from the blood vessels. Even dead lepra bacilli injected into the skin cause lesions like the tuberculoid. It should therefore not be expected that lesions of this nature will heal up quickly under sulphones. In more succulent tuberculoid lesions containing more bacilli and (like those described by RYRIE as occurring in the Chinese in Malaya), tending to pass on into the lepromatous type one would expect more valuable results with sulphones.

## PROPHYLACTIC VALUE OF SULPHONE TREATMENT

Whatever be the ultimate therapeutic effects of sulphone therapy, whether complete and lasting cure can be effected in all or in a great majority of patients, there seems no doubt that in most cases, whether in 2 or 3 years or even in a shorter period, the bacilli in the skin and the mucous membrane of the nose diminish till they become difficult or impossible to find. The presumption therefore is that cases become within that period less and less capable of transmitting infection. Also the hope of improvement through treatment inspired by the news of other patients should help to break down the tendency to concealment which often has disastrous results in spreading infection in the family and to neighbours and other associates.

Another difficulty that may be lessened is that of providing sufficient accommodation for inpatients in leprosanaria, where the average duration of residence at present often extends over several years. Speeding up the turnover, as patients recover or at least change from open to closed cases more

rapidly should add considerably to the available accommodation. This may be further increased if it is found that, instead of retaining patients in leproseries for the full period of treatment, the residual period may be spent at home under due precautions.

The discovery of a specific form of treatment has not as a rule been effective in controlling an epidemic. Witness, for instance kala-azar in Bengal and Assam, or malignant tertian malaria. In these two diseases, however, an insect vector complicates the issue. In leprosy on the other hand there seems little doubt that transmission is direct, at least in the great majority of cases. It will be of interest to see whether leprosy can be eradicated from a circumscribed endemic area through the agency of sulphones—that is to say from an area in which the existing public health arrangements had previously been unable to control the disease.

#### NEED OF PERSONNEL

Lastly I wish to make it clear that though leprosy is not as a rule quickly fatal, and perhaps just because of its low fatality it causes probably more distress and suffering in the aggregate than any other disease. So far the absence of an effective remedy for the more severe lepromatous form of the disease has discouraged personnel from engaging in anti-leprosy work. It is to be hoped that the brighter prospects which sulphone treatment has brought will attract more doctors to engage in the campaign against leprosy.

The sulphone treatment of leprosy has, as this paper has tried to show, raised a number of interesting and important questions which have to be solved. Which is the most effective form of sulphone? What is the most effective and least dangerous dosage? Can yet more effective forms or combinations be found? What is the action of these drugs? Can the low resistant type be changed into the high resistant as shown by the lepromin test? What is the length of treatment and the danger of relapse? Does drug-resistance occur and, if so, can it be prevented or remedied? These and many other problems await solution. It may be that as work on tuberculosis led to the first use of sulphones in leprosy further work in leprosy may shed light on the therapeutics of tuberculosis. Be that as it may this new form of treatment has opened up abundant scope for laboratory, clinical and field investigation, and it is to be hoped that workers in this field will not be wanting.

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In India ROGERS and MUTR (1940) found that the eyes were affected in 5 to 10 per cent. of cases and in the Out-patients Department in the Calcutta School of Tropical Medicine, where large numbers of early cases are seen, eye complications are comparatively uncommon. The low figures for India are due to the large proportion of mild cases and to the less concentration of leprosy on the face in a climate where few clothes are worn and consequently both primary and secondary lesions are more equally distributed over the whole body.

In the Albert Victor Leper Hospital Calcutta, the author (KIRWAN 1927) found out of 155 indoor patients 36 per cent. in European and Anglo-Indian patients and 20 per cent. in Indian patients showed ocular complications. These figures include the eye and its adnexa and the cases were of long standing duration, varying from 5 to 50 years with an average duration of 9 years.

NEVEZ in Kashmir found eye complication in twenty out of eighty lepers in the State leper asylum. DE SILVA (1908) working in Siam found 101 cases of ocular complications out of 500 lepers. PINCKERTON (1927) working in Hawaii found 323 cases of ocular complications including the appendages out of 363 cases. Forty were therefore unaffected. LOPEZ in Havana found that every single case of leprosy showed some lesion of the eye or its anexa at some part of its course. WOOD (1925) in South Africa found ocular complications in some form in nearly all cases. CHANCE (1916) in Norway found ocular involvement in 90 per cent. of patients who had lepromatous leprosy and in 75 per cent. who had the neural type. He is of opinion that the incidence of ocular symptom bears no relation to the duration of the general disease but may come on at any time in its course though it is not found usually until several years have elapsed. HARLEY (1946) in Panama found ocular involvement in 90 per cent. of leprosy patients not including the adnexa. The 10 per cent. of eyes free from the disease were found exclusively in recent cases.

PERCENTAGES OF OCULAR COMPLICATIONS RECORDED IN LEPROSY

Authors	Country	Percentage	Authors	Country	Percent age.
ROGERS & MUTR (1940)	India	5-10	KOGALOW	Russia	43-57
KIRWAN (1928)	India	20 Indian patients, 36 European and Anglo-Indian	KAUREN	Norway	55-80
			GHOTOCTU	Japan	75
			BORTHEN	Norway	83
DE SILVA (1908)	Siam	20	PINCKERTON (1927)	Hawaii	88
NEVEZ (1900)	Kashmir	5	HARLEY (1946)	Panama	90
BODNET	Hanoi	30	LOPEZ	Cuba	100
JEANDELAK	France	42			



of corneal sensitivity. Once leprous lagophthalmos is established it is permanent and will not recede, but despite the cornea being so much exposed, it is remarkable how so many of these cases escape corneal ulceration over a very prolonged period often extending into many years.

The lachrymal gland may occasionally be the site of leprosy but there is no disease of the lachrymal sac or duct peculiar to leprosy. Chronic dacryocystitis is, however, rather common, is secondary to the septic process present in the nose and frequently gives rise to purulent conjunctivitis and blepharitis.

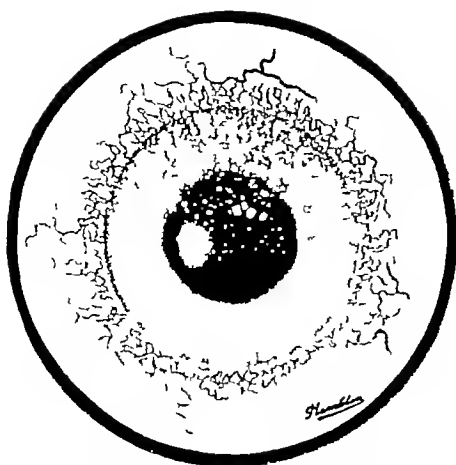
#### THE CONJUNCTIVA, EPISCLERA AND SCLERA.

*Bacillus leprae* has been found in the conjunctival secretion and in scrapings from the conjunctiva in clinically healthy eyes. Hyperaemia and congestion of the conjunctiva frequently occur. These may be diffuse or limited to a triangle on either side of the cornea. Leprous nodules are never found on the conjunctiva. The conjunctiva, like the skin, being an exposed tissue can be infected directly or more commonly the infection spreads from the surrounding skin lesions and is transported by either the lymphatics or the nerves. The bacilli do not proliferate in the conjunctiva, but in the episcleral tissue close to the sclero-corneal junction where the anterior ciliary nerves penetrate and appear to have a marked preference for this area on account of its peculiar structure and position. Yellowish gelatinous nodules appear in this area more usually on the temporal side. They extend around the limbus producing a low rampart of granulation tissue and eventually obliterate the whole contour of the limbus. The infiltration spreads into the superficial layers of the cornea destroying Bowman's membrane and produces a sclerosing keratitis. An anterior staphyloma of the cornea may result. Anaesthesia or hyoaesthesia of the conjunctiva occurs in the lepromatous form and is of great value in diagnosis as if it is found present it is a warning of impending serious ocular lesion.

#### THE CORNEA

The cornea is the most vulnerable of the ocular tissues and is very commonly involved. All varieties of keratitis are seen and can best be classified into primary and secondary. The primary ones comprise five groups—pannus, sclerosing keratitis, superficial punctate keratitis, deep or interstitial keratitis, and leproma of the cornea. The secondary ones comprise the ulcerative group which may occur in paralytic lagophthalmos and when there is loss of sensitivity of the cornea.

Pannus in leprosy is very common and presents itself in the form of a net, the meshes of which are composed of uniform branching blood vessels in contradistinction to the pannus of trachoma in which the new blood vessels



LEPROSY—SHOWING CHARACTERISTIC PANNUS AND SUPERFICIAL PUNCTATE KERATITIS



LEPROSY—SHOWING PLASTIC IRIDO-CYCLITIS WITH MILIARY LEPROMAS ON THE IRIS





are terminal and arranged in the shape of bundles. It is seen in all stages from early vascularization in the upper third of the cornea to the severe forms in which there is deep vascularization as well. Grave forms are seen causing a partial or even complete hyperplastic keratitis.

Leprous sclerosing keratitis is quite a common occurrence, in which the sclerotic coat of the eye appears to invade the cornea. It occurs as a band of variable proportions with a very pronounced white colour and new blood vessel formations are either absent or scarce. Superficial punctate keratitis is probably the most common ocular lesion in leprosy, is quite unlike other types of superficial keratitis and its presence is pathognomonic. It usually begins at the superior limbus as a light milky haze, punctuated by tiny white spots resembling grains of chalk and varying in size. These spots are miliary lepromas. The keratitis is accompanied by superficial vascularization and is not a true superficial one. At the limbus it affects the deeper layers, but tends to become superficial as it spreads into the centre of the cornea. The lower margin is delineated by a wavy line above the pupillary centre of the cornea so that vision at first is not impaired, but when the keratitis extends over the whole cornea the vision will be seriously and permanently affected.

Interstitial or deep keratitis begins at the periphery of the cornea from extensions of pre-existing lesions at the limbus and the episclera. With the corneal microscope the opacities can be seen to be composed of small nodular infiltrations with new blood vessel formations below Bowman's membrane in the superficial layer of the substantia propria and not affecting Descemet's membrane or the corneal endothelium. When it is limited to one sector of the cornea, vision is not much affected, but if it involves the whole of the cornea the vision will be permanently lost.

Another type of interstitial keratitis sometimes seen is the degenerative circular keratitis that extends around the entire corneal circumference. It simulates the peripheral annular lipid infiltration of the corneal stroma so commonly seen in elderly people who have lived in the tropics and known as arcus senilis. The superficial and deep forms of keratitis are often seen in combination and are usually bilateral.

A leproma of the cornea is never primary, but commences at the limbus and in its early growth into the sclera and cornea resembles a pterygium carnosum or vasculosum. It may be solitary and grow to a large size even impeding the closing of the eyelids. It is smooth, reddish in colour and the conjunctiva over it is adherent and mildly congested.

#### THE IRIS AND THE CILIARY BODY

Second only to the cornea, the iris and the ciliary body are the parts most frequently involved and involvement of these tissues is the commonest cause of blindness in leprosy.





Three types may appear —

(1) *Miliary lepromas* on the anterior surface of the iris, usually accompanied by the co-existing changes of superficial punctate keratitis, are the most frequent occurrence. They are greyish-yellow pedunculated or flat, pin-point bodies scattered irregularly on the iris and on the exudates on the anterior lens capsule. They are characteristic and pathognomonic of leprosy and are liable to be overlooked unless the eye is examined with the corneal microscope. The signs of plastic irido-cyclitis are often present as well—keratic precipitates on the whole of the corneal endothelium, exudates on the anterior lens capsule, irregularity of the pupil, and points of posterior synechiae. All the greater manifestations of irido-cyclitis may be observed—posterior synechiae with seclusion and occlusion of the pupil, secondary glaucoma, vitreous and lens opacities, hypotension, retinal detachment and atrophy of the eyeball. In the early stages the eye is relatively quiet and the only symptom is the gradually decreasing visual acuity.

(2) *Nodular lepromas* are much less common. They are yellowish in colour, globular, sometimes flattened, generally isolated, assume variable dimensions and have no fixed site.

(3) An acute diffuse plastic irido-cyclitis is not very common and is similar to the ordinary non-specific acute irido-cyclitis. It may occur due to the lepra reaction in the eye and may be unilateral or bilateral. It is accompanied by severe pain, lachrymation, photophobia, circumcorneal injection, extensive posterior synechiae and exudations into the pupil and vitreous body. It usually results in a considerable loss of vision.

In all varieties of irido-cyclitis, atrophy of the iris is found present in varying degrees. The whole tissue gradually becomes dull and grey, the crypts disappear, the blood vessels become visible and finally holes make their appearance.

#### LESIONS OF THE POSTERIOR SEGMENT

Although ocular leprosy is characteristically a disease of the anterior segment of the eye, there is no doubt that lesions of the posterior segment behind the ora serrata do occasionally occur in grave ocular lesions by direct spread from the episclera, limbus, iris, ciliary body, anterior choroid, ora serrata and backwards. This has been proved by histopathological examination and there is no anatomical or physiological reason why lesions should not occur. The wonder is that they are not more frequently observed. Cases have been reported in which lesions such as choroiditis have been observed by ophthalmoscopy before the anterior segment of the eye has become involved. Also involvement of the optic nerves has been found as an early and only sign of ocular leprosy. At the same time it must be remembered that *Bacilli leprae* have never been found in the choroid in histological examination and

it is possible that the choroiditis and optic nerve lesions may be due to other concomitant causes such as syphilis or tuberculosis. Personally I have never seen by ophthalmoscopic examination a lesion in the fundus behind the ora serrata which I considered to be due to leprosy.

#### PATHOGENESIS OF EYE LESIONS

The portal of entry for ocular invasion in leprosy is still in dispute. The exogenous passage is probably the most common one in which the bacilli are transported from the lepromata or maculae on the face to the conjunctiva, episclera and anterior segment of the eyeball. There are, however, points in favour of the endogenous passage in which the uveal tract is first affected, beginning in the angle of the iris and spreading in front to the iris, inwards to the ciliary body, backwards to the choroid, and outwards to the sclero-corneal limbus.

#### TREATMENT OF LEPROSY OF THE EYE

In leprosy, the loss of vision is caused by changes in the cornea and plastic irido-cyclitis. Treatment is fraught with disappointment except in the early cases, but even in the grave cases much can be done to delay the onset of blindness. The general treatment is of primary importance, but I shall here only discuss the local treatment.

Care should be taken to protect the eye from trauma. This is best carried out by the wearing of tinted glasses. Pannus is best treated by peritomy and helps to prevent the spread of corneal opacification. Lepromas at the limbus are amenable to local therapy unless the process has penetrated too far into the eye. The usual treatment of these consists in surgical measures, the application of solid carbon dioxide and diathermy, but probably the best treatment is irradiation by the Grenz rays. The most effective doses applied at one sitting are from 700 to 1,200 r, the total amount varying from 5,500 to 11,600 r. No damage to the cornea, lens, or deeper structures of the eye results from these exposures. Subconjunctival medication has been widely employed, but in my experience it is of very little use.

For corneal ulceration in lagophthalmos a tarsorrhaphy must not be delayed. As soon as the iris becomes involved the pupil must be kept dilated with 1 per cent atropine drops. Patients often suffer from atropine irritation, so it must be used with discretion or can be replaced by  $\frac{1}{2}$  per cent hyosine drops. Unfortunately many of the patients one sees show the iris bound down by posterior synechiae, the pupil small and filled with exudate so that atropine is of little use. If the pupil refuses to dilate sufficiently or if secondary glaucoma occurs a broad iridectomy should be done. The leprosy eye, despite the chronic inflammation, stands surgery very well. For muco-purulent conjunctivitis and inflammation of the eyelids penicillin drops 2,500 units per

ml. and oculentum penicillin 1,000 unit per gramme are excellent. For chronic dacryocystitis, the lacrimal sac should be removed.

### SUMMARY

The ocular complications of leprosy are the most serious lesions in the disease and occur in a very high percentage of cases. Leprosy is one of the great causes of blindness in the world. I would make a plea that every leprologist should have a knowledge of ophthalmology. The slit lamp with the corneal microscope and the electric ophthalmoscope are indispensable in the early diagnosis and recognition of the type of ocular leprosy and so every leprosanium should be provided with these articles of equipment and the medical officer in charge should have a knowledge of their use.

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## PAPER

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# THREE CASES OF LEPROSY TREATED WITH DIASONE

BY

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Among a number of cases of leprosy I have seen during the last dozen years, three have remained under my close personal supervision to date. All are open lepromatous cases of the disease. Two attend regularly as out-patients, and the third is in hospital. These three patients have been treated with diasone, and are the subject of this communication.

Until very recently diasone has been extremely difficult to obtain in this country. I was indebted to Dr Muir for my earliest supply, subsequently I was able to get a limited quantity gratuitously from the makers in the United States, and only very recently has a permit been granted to import the drug into the United Kingdom.

The dosage of diasone selected was based on the observations by PETER and PRENZLAU (1944), who considered 1 gramme of the drug by the mouth daily to be an adequate dose. With this they obtained blood levels of from 1.5 to 2 mg per cent (calculated as diasone), they did not obtain a proportionate increase in the blood levels on increasing the dosage beyond this point. FAGER and POGGF (1945) with a dosage of 1 gramme of diasone daily reported the appearance of toxic manifestations, of which haematuria, gastritis or a haemolytic anaemia were the most prevalent, these in some cases called for a suspension of treatment. It was found that a gradual build-up to this level of dosage over a period of some weeks reduced the incidence of toxic side effects and enabled tolerance to the drug to be established. COCHRANE (1947), in a letter to the *British Medical Journal*, states that diasone, "in the dosage recommended," is very liable to precipitate lepra reaction, often extremely severe. He found it impossible to maintain adequate blood levels ('5 mg per cent') with this drug. COCHRANE found sulphetrone to be the least toxic of the sulphones and a preparation with which satisfactory blood levels could be obtained.

In my cases the dosage of diasone was raised over a period of 3 or 4 weeks to 0.3 gramme by the mouth thrice daily. Iron was given concurrently throughout the course of treatment. A close watch was kept on the blood



picture and when the haemoglobin fell below 80 per cent. liver injections were given. There were temporary and trivial digestive disturbances in one case only the cause of which was doubtful. The urines were examined regularly throughout treatment, but no abnormalities were found. There was no evidence of lepra reaction in any of these cases and the general health has remained good, and indeed has improved to date. The psychological benefit derived from the treatment has been remarkable and all three patients are convinced they are improving.

#### CASE A.

An Englishman who had contracted leprosy probably in Malaya, where he had been treated since 1941 in a leper settlement. When I saw him in 1942 he had a number of macules on the trunk and limbs. He was treated with chaulmoogra derivatives by intradermal infiltration for a period of 3 years without obvious benefit. During this time his condition steadily deteriorated and there was a steady extension of the skin lesions. By early 1947 he had very numerous, extensive and flamboyant macular lesions and some nodules over the whole of the body and he had developed an early leonine countenance. He had a marked conjunctivitis of both eyes.

In April 1947 he was put on diasone, and this has been continued to date. Within 6 weeks of beginning diasone treatment there was objective improvement in the lesion and the conjunctivitis had cleared. Within another 2 months the facial appearance had returned to normal. Many of the more recent and smaller macules on the body had vanished. The older and more extensive lesions, though still evident were considerably less prominent and the nodules were smaller. This objective improvement has been sustained and, although there are still many macules to be seen on the trunk and the limbs, they appear to be further retreating.

At the commencement of diasone treatment in April, 1947 very numerous organisms could be recovered from all the lesions, and from the face. In December 8 months later there were still great numbers of organisms to be found in all areas. Many bacilli could be found in the apparently normal skin of the face.

#### CASE B

This man an Englishman was marine engineer serving in a vessel trading around the coasts of India. He states that he never lived ashore in the East. I first saw him in 1936 and found him to be suffering from leprosy. At that time he had a few lepromatous macules on the face on the chest and on the arms. There were also some very early secondary neural changes in the hand and the feet. He was treated with several courses of Reemsterma's serum over a period of 2 years without obvious benefit. Subsequently he had



while taking 0.9 grammes diasone daily. These were found to range from 1.0 mg. per cent. to 2.2 mg. per cent. (calculated as diasone), at various times while on this dosage. About a third of the drug taken could be recovered from a 24-hour specimen of urine.

#### SUMMARY AND CONCLUSIONS.

In general terms, therefore, treatment with diasone of these three cases of rapidly progressive lepromatous leprosy would appear to have caused marked retrogression of the skin lesion though it has not resulted, as yet, in any obvious diminution in the number of bacilli in the affected skin areas. There have been no gross toxic side effects attending the use of the drug and there has been no evidence of activation of the disease, in the form of lepra reaction.

In conclusion, may I direct attention to the lamentable lack of amenities for dealing with persons suffering from leprosy in this country. Apart from one small institution in the South of England no hospital will willingly or knowingly admit a case of leprosy. Cases that I have seen with few exceptions, have been hospitalized for surgical or other treatment on my personal responsibility only by concealment of the diagnosis. The exceptions have been aliens notified to the local authority. These authorities, after failing to pass the patients on to some other body have forcibly incarcerated the individuals under rigorous detention in isolation, whether this is necessary or not without the benefit of expert supervision or treatment. In some cases suicide, or death from despair has to my personal knowledge resulted from such measures during the last few years in the North of England. It is hardly to be wondered at that concealment is practised by patient and by doctor alike. It is high time a more humane and enlightened attitude in regard to leprosy should obtain in this country. Facilities for hospitalization under proper skilled attention should at least be available when needed to those suffering from the disease.

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## DISCUSSION

The President (Sir Philip Manson-Bahr) said they had heard and appreciated three papers on leprosy, presenting an aspect of the disease which few of those present had so far realized. All three papers were of very high standard and, so far as he knew, nothing like them had appeared in the literature of any country. All present would sympathize with Dr ADAMS's experiences, and everybody in this country who had treated leprosy would agree with him. He, himself, had seen similar tragedies in his practice in London in years gone by. The public attitude towards this disease was deplorable. The fact of leprosy being regarded with such horror by the people of this country was one of the results of our early upbringing. The meeting was fortunate in having General MACARTHUR to speak now on this aspect of the subject.

Lieut-General Sir William Mac Arthur gave an interesting account of the many misconceptions which had arisen regarding the incidence of leprosy both in this country and elsewhere in mediaeval and even in Biblical times. He showed how these were largely attributable to the too literal interpretation of words which in early days had a wider and more general application than they now have.

Dr W E Cooke I have little to add to the very excellent papers we have had tonight, but I have had the very good fortune to see the results of the diasone treatment of patients at the St Giles' Homes. When Dr MUIR came back from Trinidad we had only one or two persons who were unwilling to take the drug, but, seeing how the others progressed, eventually, every patient wanted it. I congratulate Dr MUIR on the results, and I am prepared to substantiate what he has said about our patients in the Homes.

We have been fortunate in getting Colonel KIRWAN's help. When I heard he had come back to work in London I invited him to come to the Homes to see our patients. The majority have eye lesions and some are blind. This, I believe, could have been avoided had they been seen sooner by an ophthalmologist, expert in leprosy of the eye. Under Colonel KIRWAN's care their eye conditions have improved and the sight of some has been saved.

We have an average of ten to twelve patients in the Homes, and since we began the diasone treatment we have been able to allow four patients to leave the Homes and report periodically for observation. Four patients so discharged in  $2\frac{1}{2}$  years is a good average out of the small number we have. Among these patients is one who had objected strongly to taking the diasone.

I would like to support Dr ADAMS's observations about the need of more accommodation for lepers in this country. More of these unfortunate people

are coming here having acquired the disease probably as the result of too long residence in the East under war conditions, but one factor that brings them is that leprosy is not notifiable in this country. During the last 2 or 3 years I have seen at least a dozen of these patients and our accommodation at the Homes is inadequate for such numbers. We admit them as soon as possible but our beds are full now and I know there are some patients in the offing we would like to take.

We suffer from one disability—the St. Giles Homes are not a hospital. The two local doctors who attend the Homes do excellent work, nobly supported by the nursing sisters of the Community of the Sacred Passion, but they cannot carry out full hospital treatments. The Homes were originally opened as a refuge for these patients, not as a hospital and owing to various factors I do not consider they are suitable to be transformed into a hospital in the future.

We do not admit aliens, and this also has been a difficulty but here the Seamen's Hospital Society has come to the rescue of some of these alien patients by admitting them to one or other of the hospitals in their group but others have been left to shift for themselves. The time has come when leprosy should be notifiable, and the Government should institute a place where these unfortunate people could be kept under supervision and be properly treated.

Dr G Brownlee I welcome the opportunity to add something to this discussion of the chemotherapy of leprosy. My concern is with the pharmacology and therapeutics of sulphetrone, and I have been very much interested by Dr Nuts's remarks on the anaemia caused by this drug.

We have recognized in pharmacological studies of sulphetrone three distinct anaemias in the experimental animal, and in man. The observations are recorded in a paper at present in the press. Although applied specifically to sulphetrone, the observations are probably applicable to promin and dissonne. First, there is a hypochromic anaemia, due to the capacity of sulphetrone to combine with alimentary iron—it has been demonstrated that the iron complex made by Mr W. H. GRAY is not absorbed. Secondly there is a nutritional anaemia which arises in an interesting way. It can be shown in the experimental animal and man that sulphetrone markedly modifies the bacterial flora of the gut. An immediate effect in the animal, and by close analogy and some allied work, in man is to cut down the biosynthesis of vitamins of the B-complex group. The nutritional anaemia which results may be prevented, or cured by the administration of a suitable yeast preparation. It is interesting to note, in this connection, that toxic blood levels of sulphetrone eventually result in interference with the oxygen-carrying capacity of the red cells when it is remembered that nicotinamide known to be produced by biosynthesis, enters into the composition of the coenzymes involved.

BROWNLEE, G. GREEN A. F. & WOODWINE, M. (1948) (In press) *Brit J Pharmacol.*

The discovery that yeast prevents one type of anaemia due to sulphone therapy has also been made by workers in America with both promin and diasone, but I think this is the first time it has been put on a sound experimental basis

Even when sulphetrone is administered simultaneously with iron and yeast, there is a residual haemolytic anaemia which is associated with a red cell fragility. It is always present and may be large enough to reduce the haemoglobin concentration to the equivalent of 60 per cent (Haldane) in patients with blood sulphetrone concentrations of 7.5 to 10 mg per 100 ml. After 5 years' experience of treating tuberculous patients with sulphetrone, we have to confess that we are no longer much concerned with this resistant haemolytic anaemia. It appears to stabilize at about 60 per cent. Indeed the red cell fragility has an interesting effect. In tuberculous patients the production of primitive red cells is depressed so that the ratio of primitive reds to whites is abnormal, under sulphetrone therapy the production of primitive reds is stimulated so that the ratio again becomes normal. As may be inferred from the foregoing observation, sulphetrone has no demonstrable effect on white cells.

There is a second aspect of Dr Muir's review on which I should like to comment, this is dosage. In the treatment of tuberculosis we have adopted a higher dose-level than has been advocated for leprosy. Indeed, the dose has been the highest tolerated level of about 10 mg per 100 ml. I appreciate and approve his caution and realize the difficulty, peculiar to his patients, of lepromin reactions. The approach in tuberculosis was by way of the effective blood concentration in experimental infected animals, and then to the highest tolerated dose in man. This experimental approach is denied us in leprosy and caution is justified. However, I have an uneasy feeling that the dose levels may prove to be rather lower than they might have been. It may be that experience with previous, more toxic, sulphones is to some extent responsible.

Mention has been made of acquired drug-resistance of Hansen's bacillus, this possibility is always associated in the mind of the experimental worker with inadequate concentrations. It is a constant fear in chemotherapy that one is using a sub-optimal dose and it is a point that must have occurred to many of us in connection with the low doses named today. Development of resistance to *M. tuberculosis* has been recorded with sulphetrone after a year's continuous therapy, but it has not become a problem.

I think, Sir, that these are the main points that occurred to me when the two speakers were referring to these drugs. May I thank you again for giving me the privilege of speaking.

**Dr C C Chesterman** On my first leave from the Congo I visited our old family physician, and told him that up to 5 per cent of the people there were lepers and that we ought to do something about it. He replied, "You'll never cure leprosy, it is taken as a type of sin in the Bible." In defence of the

Old Book, I would remark that it contains much which is a warning of what not to believe as well as what to believe.

I had the privilege of being in Nigeria 18 months ago when Dr DAVEY made the first evaluation of diosone treatment and it was amazing to see the interest, enthusiasm and new hope generated. Six months ago I was in Carville, near New Orleans, and I saw the leprosanum there and learnt the story of how Dr ERICKSON brought back the bacon." The reference was to the Centennial Meeting of the American Medical Association, in which the Carville exhibit of the result of the sulphone drugs was awarded the silver medal. The whole camp was thrilled by the report and the outlook captivated the community. They were very anxious to get rid of segregation now there was hope of curing leprosy. It should not be regarded as worse than tuberculosis. Now there is to be no more need for voluntary gifts to finance our hospitals, one object of charity might be the provision of means for the care of lepers.

Mr W H Gray I can add a brief historical note. Not all the sulphones that have been mentioned tonight are derivatives of 4,4'-diaminodiphenylsulphone, but that was the first to be brought into trial in antibacterial chemotherapy. It had been known, like sulphanilamide long before—by a coincidence although in quite unrelated fields, the two were first isolated in the same year—but in 1937 in the early days of sulphonamide therapy it was obtained as a by-product in sulphanilamide manufacture at the Wellcome Chemical Works\* and found to be very much more active than sulphanilamide against experimental bacterial infections in mice.

Unfortunately it was also a good deal more toxic, and Dr HENRY and I took over the task of detoxicating it, and were able to prepare derivatives of much lower toxicity including the sulphonate now known as sulphethione (Wellcome Foundation, Ltd., Henry and Gray British Patent 491,265).

Dr E Muir I would like to express my appreciation of Colonel KIRMAN'S paper and the valuable work he is doing on the eyes in leprosy. I must also express my appreciation of the remarks of General MACARTHUR. I think he has done a great deal to remove the misapprehensions and false ideas that so many people have with regard to leprosy. I would like to emphasize again what Dr ADAMS said about the need of more accommodation for people with leprosy in this country. Even this last year there have been far more people with leprosy found in this country coming from the tropics than ever before in my experience, and I think the matter is becoming more and more urgent every day. Dr BROWNLET'S remarks on the anaemia was of very great value to the discussion also what he said about dosage. I would like to express my thanks to the various contributors to the discussion.

BUTTLE, G. A. H., STEPHENSON, DORA, SMITH, S. DREW, T. & FORTER, G. E. (1937). *Lancet* 1: 1331.

**Colonel E W O'G Kirwan** There are no questions for me to answer. I think it is not generally known that leprosy is one of the great causes of permanent blindness. Much could be done to prevent that blindness if we could only treat the disease early and in favourable surroundings.

**Dr A R D Adams** I have nothing to add, except to stress that there should be adequate provision for persons suffering from leprosy in this country. We do not know what number of cases of leprosy there are in Great Britain. I saw the figure 250 in print some years ago, but this is a pure guess, and there may be either 50 or 250. Whatever the number was at that time, I think it very probable that it has doubled within the last few years for the reasons suggested by Dr Muir.

**The President** There remains little for me to say except that I should like to see something concrete come out of the various expressions of opinion made known to us tonight. Dr ADAMS, Colonel KIRWAN and Dr MUIR all advocated the necessity of a more sane attitude towards this disease. On three occasions in my life I have been present with Dr MACLEOD when a direct approach was made to the Ministry of Health, trying to induce them to make some public statement about it, or provide a hospital or part of a hospital where these cases could be sent. I have seen a good many in my consulting room. Some were in high ranks of society wandering about London. Another was a man who ran an ice-cream shop and sold it openly. His was a terrible case with numerous nodules in his skin, and exuding bacilli from his nose. But nothing can be done, because directly you try to do anything you get yourself and the patient into trouble. You are in duty bound in charge of your patient and have to protect him from the publicity to which he might be exposed. Is it possible that we could send some recommendation to the Ministry of Health under this new scheme that we are now about to see? We might recommend that some hospital, or part of a hospital in London, should be set aside for the treatment of leprosy, as we now know that the outlook is more hopeful than ever before. Many years ago at the Albert Dock Hospital, in MANSON'S day, there was an annexe into which lepers were put. There was somewhere to send patients and know they would get sympathetic treatment from the sisters and doctors who dealt with the disease, but nothing of the sort has been available since the Albert Dock Hospital was given up as a centre for tropical medicine in 1928. Could not Dr MUIR get together some public men who would stress this point with the Government of the day?





## COMMUNICATIONS.

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### THE DEVELOPMENTAL CYCLE OF *HEPATOCYSTES* (*PLASMODIUM*) *KOCHI* IN THE MONKEY HOST

BY

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#### INTRODUCTION

A brief description (GARNHAM, 1947) has been given of the mature schizogonic stage of *Plasmodium kochi*. The present paper completes the study and gives an account of the full cycle of the parasite in the liver.

It is not proposed to discuss here the literature regarding *P. kochi* which, in spite of the clarification achieved by SINTON and MULLIGAN (1932 and 1933), and by HAWKING and HUNT (1947), still remains confused. The former authors define *kochi* in the following terms —

- 1 It is the most usual *Plasmodium* found in the blood of monkeys of the genus *Cercopithecus* and also in *Papio*
- 2 It has little pathogenicity
- 3 Gametocytes are the commonest forms in the peripheral blood
- 4 Schizogony appears to take place in the internal organs
- 5 There is no enlargement or stippling of the infected cells
- 6 Gametocytes lie free in the plasma. Their nuclei are large, vesicular, and poorly staining with compact chromatin
- 7 Transmission of the infection to clean animals is unsuccessful

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\*This investigation was carried out by means of a grant from the Medical Research Council, in accommodation kindly provided by Professor H. E. SHORTT in the Department of Parasitology, London School of Hygiene and Tropical Medicine. The author is much indebted to Professor SHORTT, Dr C. M. WENYON and Dr C. A. HOARE for advice and criticism in regard to the interpretation of the developmental stages of this parasite and to its systematic position. He also gratefully acknowledges the help of his late colleagues at the Medical Research Laboratory, Nairobi, in the earlier stages of the enquiry. Dr F. HAWKING very kindly placed at the author's disposal facilities and *kochi* material in the National Institute of Medical Research, Hampstead. Special acknowledgment is made to Mr W. COOPER, of the Department of Parasitology, London School of Hygiene and Tropical Medicine, for the paintings reproduced in Plates I and II.

Practically all workers have failed to find the schizonts of this parasite. SCHWENK (1933) however described and illustrated some forms which he called "*corps plasmodiques*" which he found very rarely in the bone-marrow and in the peripheral blood. These resembled to some extent the smears from merocytes, of "*schizonts*" found by myself. LIVANOFF and SCHWENK (1932) were undoubtedly dealing with the mature liver forms, which they described as a new parasite under the name of *Hepatocystis simiae*.

### MORPHOLOGY OF GAMETOCYTES

I have studied keds in Kenya and Uganda from 1934 to 1947. This material has been compared with preparations from Tanganyika (of Dr FAIRBAIRN), from the Belgian Congo (of Professor VAN HOOF) and from West Africa (of Dr HAWKINS). No points of difference were observed. The following is a brief description of the forms found in the peripheral blood.

The youngest parasite consists of a minute dense spot of chromatin with a tiny loop of cytoplasm, the ring being rather smaller than the earliest form of *P. falciparum*. With growth, the chromatin tends to spread out into a semi-circle or into multiple dots. Later a large amoeboid ring with a vacuole develops, an appearance which is contrary to the usual idea of a young gametocyte which is stated to have no vacuole and little amoeboidicity. These forms are not uncommon. There is no stopping of the red blood cell. Pigment forms early though in such tiny particles that it is difficult to see it. The cytoplasm then grows out into wedge like pieces which gradually fill in, until a spherical body is produced. This occupies all the erythrocyte and when mature is slightly larger than it. All these forms are gametocytes as was proved on several occasions by incubating defibrinated blood at 37° C. with a few drops of 50 per cent. glucose solution. The ring forms grew into typical gametocytes. Development from the young ring to the mature gametocyte takes about 4 or 5 days (a period confirmed by *in vivo* observations). In two instances, the culture on the 6th day showed an enormous accumulation of mature forms, they were many times more numerous than on the day the blood was drawn. I do not know whether this was an artificial effect, or whether it represented perhaps the development of numerous merozoites inapparent in the original blood.

The mature gametocytes are very characteristic objects in a stained blood film. They stain rather poorly in comparison with other blood protozoa. With Romanowsky stains, the macrogametocyte has a steely blue colour whilst the microgametocyte is a less dense, brucine-coloured parasite. The pigment is so fine that it is difficult to determine its exact tint. The most striking feature is undoubtedly the nucleus. This consists of two portions in both male and female. In the male there is a large oval shaped pale pink area occupying one-third to a half of the parasite. Scattered in this are numerous deep red granules or threads of chromatin. The nucleus of the macrogametocyte is much smaller and is composed of a pale pink area with dense chromatin in the

middle granules The chromatin may be a single lump or be divided into up

Exflagellation is easily observed by mixing a drop of blood with saline solution and watching the preparation under dark ground illumination. At a temperature of about 70° F, exflagellation starts within 3 minutes usually over in 20 minutes. Not more than four microgametes were seen to be extruded by a single gametocyte, their length varied between 9 $\mu$  and 12 $\mu$ . On several occasions the gamete was observed to pull out a long finger process of the protoplasm of the gametocyte, an appearance also noted by ANDERSON and COWDRY (1928) in their studies on exflagellation of *P. kochi*. The infection in the monkey is very persistent. Animals kept under mosquito-proof conditions commonly exhibit parasites for as long as 15 months. For this reason it is thought that some of the merozoites of the liver merocytes must be asexual in character and give rise to successive tissue forms, thus maintaining the infection for long periods without re-infection. The parasite causes no obvious harm to the monkey, and temperature records kept for many months, both in young and in heavily infected animals, showed no evidence of a febrile response.

#### RESULTS OF SUB-INOCULATIONS

Inoculation of *kochi*-infected blood into clean monkeys gave rise to transient infections. This is contrary to the experience of most workers as noted by HAWKING and HUNT (1947), though COWDRY and COWELL (1928) also obtained mild infections following sub-inoculations of this parasite. My results are summarized below —

- 1 A *Cercopithecus aethiops* monkey (M 6), born in the laboratory a year previously and kept under mosquito-proof conditions, was inoculated intramuscularly with 2.5 c.c. of blood from a monkey (M 28) showing rings and gametocytes of *kochi*. Earlier examinations of M 6 were always negative, 3 days after inoculation it showed scanty gametocytes and these continued for 3 days. Subsequently, the blood remained negative for 6 months.
- 2 A *C. aethiops* monkey (M 7), born in the laboratory a year previously and kept under mosquito-proof conditions, was inoculated intramuscularly with 2.5 c.c. of infected blood from M 28. No parasites were found in M 7 until 5 days later, when scanty gametocytes appeared for 2 days. It was subsequently negative for 3 months.
- 3 A baby *C. mitis* monkey (YY), previously blood negative, was inoculated intramuscularly with 3 c.c. of blood from an animal showing numerous rings and gametocytes of *kochi*. Parasites were first seen in YY a week later—they were very rare (about one in fifty fields of a thin smear) and persisted on and off for 3 weeks.
- 4 A young *C. aethiops* (U 736), repeatedly negative for *kochi*, received 1 per cent blood intraperitoneally from Monkey LL (parasite density approximately 1 per cent of RBCs) for 8 days. Scanty gametocytes appeared in U 736 on the following day and lasted for 8 days. It was then negative for 37 days, when it received 3 c.c. of *kochi*-infected blood from MKRR. Next day the blood showed parasites again chiefly small solid forms, and on the following day larger forms. It was then negative for a week, when it was sacrificed and the liver was examined. No merocysts were seen.

5 An adult *C. arthrops* (K) from *kochi*-free locality and parasite-free was given intramuscularly 3 c.c. of infected blood from MKRR. K showed no parasites the day after the inoculation, but scanty gametocytes were seen for the next 16 days when they disappeared. Three weeks later it was given 4 c.c. of *ascaris* blood from baboon to see if foreign blood would cause the reappearance of parasites. None were observed and the monkey was sacrificed. No merozoites were present in the liver.

6 An adult *C. satiti* monkey (1194) persistently *kochi*-negative received 2.5 c.c. of infected blood from MKRR. The following day it showed parasites—chiefly tiny oöid forms then larger forms for 2 days and afterwards nothing. It was sacrificed and the liver showed no merozoites.

7 An adult *C. satiti* (M. 8) whose blood had never showed parasites, was given 10 c.c. of infected blood of MKRR. Twelve hours later scanty mature gametocytes appeared in the blood of M. 8, and these persisted for 3 fortnights. After that the blood was negative.

The above experiments indicate that sub-inoculations result in a passive transference of parasites, including perhaps the transfer of inapparent merozoites which grow into mature forms. It is probably necessary to use a fair quantity of heavily infected blood to get positive results. Further work is obviously required for a proper understanding of this phenomenon.

#### DISTRIBUTION OF *P. kochi* IN KENYA AND VECTOR.

The parasite is well distributed in Kenya. A list of species of monkeys is given in the earlier paper and it is to be noted that baboons in Kenya have not been infected (at least by myself) though they are positive elsewhere in Africa (e.g. Nyasaland, Congo and West Africa). In Kenya *kochi* has been found in the following places: Taveta Forest (2,500 ft.) Tetia Hills (5,000 ft.), Kakamega Forest (5,000 ft.) Nairobi (5,200 ft.), Ngong Forest (5,500 ft.), Girama Reserve (sea level to 1,000 ft.) Makueta (4,000 ft.) and Kodera S.K. (4,500 ft.). In the forests above 6,500 ft. the monkeys appear to be *kochi* free.

The insect vector of the parasite is unknown. Many unsuccessful attempts at transmission have been made with different species and genera of mosquitoes. My own experience has been limited to feeding experiments with *Aedes aegypti* in Kenya, and *Culiseta* sp. in England. No oöcysts or sporozoites were found. Nearly a thousand wild caught *adefines* and *culicines* from the Ngong Forest (where *kochi* infected monkeys abound) were dissected, and no sporozoites were found in the glands. Simuliidae and *Glossina* are absent from this forest, so these insects can presumably be excluded from the list of possible vectors, as may also hippoboscids and ticks which are not (or very rarely) found on monkeys.

#### DEVELOPMENTAL STAGES

Complete postmortem examinations were made of monkeys infected with *P. kochi* and smears and sections were made of the liver, brain, lungs, spleen, heart, kidneys, inguinal lymph glands, mesentery, intestine and bone marrow.

With a single exception, the liver was the only organ which showed developmental forms. The exception was a heart smear which contained a few unpigmented "schizonts" of a similar type to those seen in the liver.

The characteristic feature of the tissue phase of *P. kochi* is the scarcity of the foci. But the scarcity is counterbalanced by the size (up to 2 mm in diameter) and by the enormous number of merozoites present in each mature merocyst. It is exceptional to find more than one to ten cysts in the liver of an infected animal. It is therefore easy to understand the difficulty of finding the younger forms which, unlike the mature ones, are invisible to the naked eye. However, one monkey exhibited a 100 or more merocysts in the liver, and serial sections made from different parts of the organ demonstrated all the early phases.

#### EARLY STAGES

The identification of the very youngest parasite is difficult, because so many intracellular granules exist in liver sections, which may or may not be of parasitic origin. In the vicinity of a ruptured mature merocyst, however, the free merozoites may be seen (Fig 7) and others within both phagocytic and parenchymatous cells. As many as thirty merozoites may be counted inside a parenchyma cell. Apparently, in most instances, development proceeds no further, possibly because the merozoites are sexual in nature and have entered the tissue cell by mistake.

Fig 1 shows the next stages, two very young parasites and two slightly older. It is possible that this picture represents the invasion of a cell by four merozoites, two of which have begun to develop whilst the growth of the other two has remained stationary. These latter bodies are about  $1\mu$  in length and appear to consist of a central mass of chromatin surrounded by a thin coat of cytoplasm. Another intraparenchymal body of a similar type was also identified in another section with reasonable certainty. Nuclear division occurs and the nuclei distribute themselves on or near the surface of the parasite in a regular peripheral arrangement (Fig 1). The smaller of the two parasites in the illustration is  $4\mu$  in diameter, the larger  $5\mu$  in length. The nuclei are very sharply defined and are round, except prior to division when they become elongated. The cytoplasm is very light and almost invisible.

As growth proceeds, the nuclei become more closely packed on the surface of the parasite. By the time it has reached  $15\mu$  in diameter, the cytoplasm begins to show up, though it is still fine and slightly vacuolated (Fig 2). Very soon after this, the cytoplasm becomes denser and presents a slatey blue, ground glass, appearance, whilst the nuclei remain peripheral in distribution. The parasite is still circular up to  $30\mu$  in diameter, but when it is that size, nuclear division seems to take place more irregularly, the size of the nuclei varies considerably and the peripheral arrangement starts to disappear, first two

circles of nuclei appearing in section and later irregular chromatin masses occurring towards the interior. The nuclei sometimes lie in a tiny vacuole.

Very striking changes are meantime occurring in the host cell (Fig. 3). Hypertrophy starts when the parasite is  $15\mu$  or even earlier. The cell is then double the normal size and the host nucleus takes up a position at one end, though remaining of normal size. Very soon the nucleus divides and four nuclei arranged in pairs at opposite ends of the cell may be seen. At the same time each nucleus enlarges to more than double the normal size. During this process of hypertrophy the protoplasm tends to shrink away from the parasite, leaving the latter surrounded by a clear halo. The protoplasm becomes condensed near the cell margin.

Up to this stage of development, the parasite is circular and the nuclei still preserve to some extent a peripheral arrangement. The next step represents the beginning of an infolding of the surface of the parasite, and it is accompanied by the multiplication of the nuclei throughout its body. At first, a depression forms on one side and this was first observed in a parasite measuring only  $27 \times 18\mu$ . This was an exceptionally early case, possibly due to the fact that the parasite was developing in an abnormal situation, in a sinusoid, instead of inside a hepatic cell. The usual size is larger—about  $45 \times 25\mu$ . The cytoplasm is now very dense, except for the vacuoles which are such typical and prominent features of the later stages. The nuclei are slightly irregular in shape and do not stain uniformly. They are scattered throughout the parasite. Further indentations in the parasite surface appear with increasing vacuolation of the cytoplasm. The nuclei at this stage bear no special relationship to the vacuoles. Their size varies, and in some parasites may be as much as  $1\mu$  across. The following measurements were obtained of three parasites at this stage of development —

$30 \times 38\mu$  (multiple but shallow indentations).

$60 \times 24\mu$  (deeper indentations).

$75 \times 75\mu$  (deep indentations with large central vacuole).

The hypertrophied hepatic cell with multiple nuclei is still easily distinguished. The nuclei are not only enlarged, but pyknotic also. At this stage, the dark blue colour of the parasite with red chromatin dots makes it a striking object in Romanowsky stained sections under a middle power of the microscope (Fig. 4).

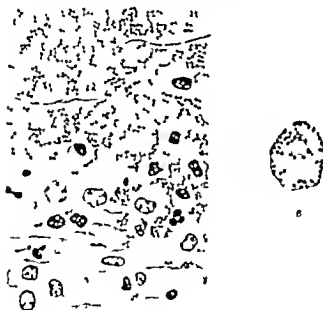
The parasite next increases its surface area, by a much more violent indentation, giving rise to an appearance resembling chorionic villi. Long fjords penetrate into the very centre of the parasite which still retains, however, a circular or oval shape. One such form measured  $105 \times 135\mu$ , another  $143 \times 75\mu$  (Fig. 5). The nuclei are now more numerous in the centre of each convolution: they are round, or oval in shape about  $0.7\mu$  across. They stain deep purplish red with Romanowsky in contrast to the deep blue of the cyto-



FIGS 1-4 Developmental cycle of *Hepatocystes kochi* in sections of liver  $\times 800$  approx

- FIG 1—Early stage showing two parasites with peripheral nuclei and two minute bodies probably representing the earliest stage Maximow stain
- FIG 2—Parasite with peripheral nuclei Maximow stain
- FIG 3—Nuclei losing their peripheral position Note the great hypertrophy of the infected liver cell, with four large nuclei Maximow stain
- FIG 4—Parasite showing commencement of invagination of surface Nuclei are now uniformly distributed and cytoplasm is becoming vacuolated Giemsa MacNamara



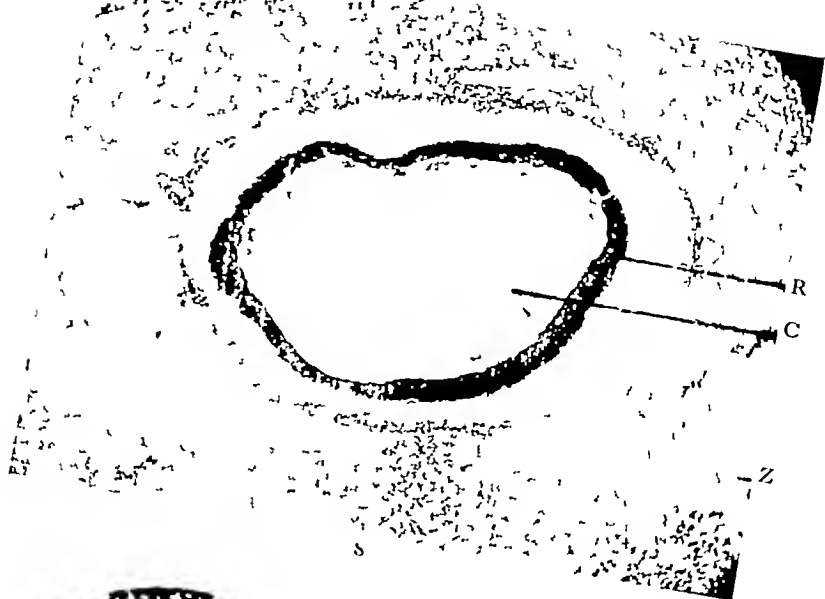


FIGS. 5-7 —Developmental cycle of *Hepatoxystus leucki* (contd.)

FIG. 5 —Half of large form with cytoplasm much convoluted. Vacuoles are now large (30) approx. (Gerrits MacNamara)

FIG. 6 —L. pigmented schizont. Smear made from merozoites fixed in methyl alcohol and stained with Giemsa. (38) approx.

FIG. 7 —Rupture of merocytes and escape of merozoites into sinusoid of liver. Some merozoites also are seen within macrophages and others inside parenchyma cells (39) approx. (Gerrits MacNamara)



FIGS 8-10 — *Hepatocystes kochi* in sections of liver Stained Giemsa MacNamara

FIG 8 — Mature merocyst  $\times 70$  approx

R = rim containing innumerable merozoites

C = colloid interior of cyst

Z = zone of reaction

The space between R and Z is an artifact caused by shrinkage in fixation

FIG 9 — Part of rim of nearly mature merocyst  $\times 700$  approx

Differentiation into cytomeres with peripheral nuclei

FIG 10 — Part of rim of mature merocyst  $700$  approx

All differentiation is lost and merozoites are packed tightly together except on exterior margin and at extremities of the finger-like processes



FIG. 11 L.—Pathological changes in the liver in *Hepatorystis kochi* infection. Sections stained with Giemsa MacNair's.

F 11 —Giant cell from zone of reaction around mature meront (740 approx).

FIG. 1 —Degenerating meront (110 approx)  
 P = Structureless remains of parasite  
 G = Giant cell layer  
 F = Fibroblast layer  
 VI = Polyblast layer  
 L = Liver parenchyma.

plasm With Heidenham's haematoxylin, no increased definition of structure was visible These forms are still apparently in the original single hepatic cell, now enormously enlarged The cell is lined by a membrane similar to that of uninfected parenchymatous cells, which was shown, by Gömöri's stain, to consist of reticular fibres Adjacent to and inside the membrane is a little granular material much like the protoplasm of the ordinary cell Then comes a clear space and in this lie the hypertrophied and distorted hepatic cell nuclei The nuclear material of these is much fragmented, though occasionally a well-defined nucleolus may be seen The margin of the nucleus is irregular and rarely circular

### MEROCYST

The final phase of development is the formation of the merocyst The central vacuole or vacuoles become distended with fluid and gradually press out the convolutions to form a thick rim to the cyst Strips of the cytoplasm are left dangling into the interior, whilst on the outside the convoluted surface is pushed in trabecular or irregular finger-like processes into the liver tissue Very often, in fixed material, the latter effect is lost, because the parasite shrinks away from the surrounding tissue The exact nature of what happens inside the parasite itself is not quite clear It was originally thought that definite cytomere formation occurred, but examination of more material has shown that clear cut differentiation into discrete cytomeres such as described by ARAGAO (1908) in *Haemoproteus columbae* probably does not take place The following stages have been traced —

- 1 The nuclei increase in size (to  $3\mu$ ), at the same time becoming less dense This appearance was seen in one part of the plasmodial rim of a nearly mature merocyst, which elsewhere contained the normal small nuclei
  - 2 These large nuclei break up into a number of smaller ones which take up a peripheral arrangement, leaving a space inside occupied by fine cytoplasm (Fig 9) These give the appearance of individual structures, but it is probable that the discontinuity is more apparent than real
  - 3 However, the cytomere effect is preserved in the next phase where the nuclei multiply, and eventually a packed mass of curving sausage-shaped bodies is produced, each of which contains many nuclei This stage resembles the illustrations of WENYON (1926) of the *Haemoproteus* schizonts in sections of kidney of the Baghdad sparrow, though the septa are missing
  - 4 Lastly, all differentiation is lost and the nuclei lie in the tightly packed mass, characteristic of the mature merocyst
- The above interpretation of late nuclear multiplication may be incorrect

It is possible that the increase in nuclear size may be a degenerative effect and that the apparent peripheral arrangement of nuclei in a cytomere really represents the distribution of nuclei around the vacuolated sponge-work of the cytoplasm. The third phase—the *Hannoprotus* effect—is, however, fairly often seen and suggests that some sort of differentiation exists. It is at least as obvious as the "cytomeres" described and illustrated by JAMES and TATE (1938) in exocytrocytic schizonts of *P. gallinaceum*.

The mature merocyst presents a most striking appearance, to the naked eye, on section under the microscope (Fig. 8) and in smears. It is most easily seen on the surface of the liver though apparently it has an even distribution throughout the organ. The cyst is of a greenish-white colour and is translucent. On puncture a clear fluid escapes, and in this fluid bits of the fragile "plasmoidal rim" can be seen as minute white flakes, which have become detached on rupture of the cyst. Smears are best prepared by making a small incision in the cyst wall, using the low power of a binocular microscope withdrawing the fluid with a capillary pipette, and smearing drops as in the preparation of blood films.

Six fully developed merocysts were measured in section after formalin fixation and the mean dimensions were  $1.8 \times 1.3$  mm. Five cysts around which tissue reaction with fibrosis was progressing actively measured only  $1.1 \times 0.8$  mm.

The interior of the cyst consists of the coagulated fluid, which with Romanowsky stains appears as a red homogeneous mass. Encircling this is the plasmoidal rim about  $100\mu$  in width bordered on the outside by a continuous wavy margin which in life lies in contact with the host tissues. The waves or less often long trabecular processes push in between the cells. This outer hyaline margin probably represents the thicker ectoplasm of the growing parasite and may have, fused within it, the remains of the original hepatic cell membrane, all trace of which has otherwise disappeared. It does not give the argyrophil reaction.

In between the wavy border and the colloid interior lie the innumerable nuclei (Fig. 10), forming the wall of the cyst. They are so close together that little cytoplasm is evident except at the extreme edge or in the dendriform processes. Most of the nuclei stop at the base of each process which contains a few scattered nuclei at the most. This cytoplasm is acidophilic, instead of the highly characteristic bright blue of that of the younger parasite. Very often there are numerous vacuoles to be seen between the masses of nuclei, and the only other structures are oval red-staining bodies which may be "residual bodies." The latter are best seen in sections of  $3\mu$  thickness or less.

Individual merocysts vary little in structure, though the appearances differ according to the plane of section. If made at a pole a solid mass of nuclei only is cut through if the section happens to go through one of the dangling strips this will show as an island in the middle of the colloid interior.

Shrinkage during fixation causes slight changes in the relationship of the structures, and the nature of the cellular reaction gives rise to different general pictures. Twin cysts are occasionally seen, probably resulting from the development of two parasites in a single cell.

Smears (Fig 6) made from mature merocysts present an equally characteristic appearance, and one that at once suggested that the cysts were part of the *P. kochi* cycle. Large numbers of unpigmented schizonts, reminiscent of *P. gallinaceum*, were revealed. The resemblance, however, is not really as close as was at first thought, and it is doubtful if these structures are properly to be termed "schizonts" at all. What, apparently, happens is that in the course of smearing, pieces of variable size of the very fragile plasmodial mass are detached and before drying, the cytoplasm rounds itself off to give an apparently definite edge to individual "schizonts". The nuclei are not all at exactly the same stage of evolution and consequently the "schizonts" present various appearances. The cytoplasm varies in colour from that peculiar intense blue characteristic of sporozoan protoplasm to such an attenuated colour that it is hardly visible. These variations perhaps represent different stages of maturity, the latter being the most mature. The smallest pieces seen in smears are less than  $4\mu$  in length, there is hardly a limit to the size of the largest fragments, though commonly, round objects, about  $40\mu$  in diameter, occur. The largest pieces are extremely irregular in shape, though in smears made from some merocysts, they assume a narrow oblong form with pointed ends. The number of nuclei varies much, in some quite large "schizonts," there may be only four or five, in others of the same size, thirty or more. Their shape, likewise, shows little uniformity, sometimes the nuclei are round, sometimes wedge-shaped, semilunar, or quite irregular. The chromatin must be very soft, because even in impression smears, it tends to become drawn out into red strands. In what are assumed to be the older forms, little or no cytoplasm is visible and this portion of the smear may consist of innumerable, apparently bare, nuclei. Such an appearance corresponds to the merozoites seen escaping from a ruptured merocyst, in section. A very characteristic feature is the presence of clear cut circular vacuoles in the cytoplasm, exactly like the vacuoles described by WASIELEWSKI and WULKER (1918) and ARAGAO (1908) in *Haemaphysalis* schizonts and by COLES (1914) in *Leucocytozoon* schizonts. It is interesting to note that such vacuoles are absent in the exoerythrocytic schizonts of avian or saurian malaria and of certain other closely related blood protozoa, e.g., the blue bodies of *Theileria parva*.

It was assumed in the earlier paper that the cycle ended in the escape of merozoites via the liver tissue into the general circulation, and this was confirmed by finding a merocyst in the process of rupturing (Fig 7). The way border bursts at one point and the merozoites first escape into the cellular reaction belt, thence into the sinusoids between the columns of parenchyma, and finally into the larger efferent vessels. This process is accompanied by

some haemorrhage, and the blood may reach the interior of the cyst, carrying merozoites with it. It is only at this stage that merozoites are seen free actually within the colloid interior. Little groups of parasites occur in the liver tissue in the immediate vicinity of the burst, but not elsewhere in the section. None of the parasites, not even those inside the large veins have yet entered erythrocytes. Phagocytosis of the merozoites is active everywhere, in the tissue reaction layer in Kupffer cells and in wandering histiocytes. Occasionally the parasites are seen inside the parenchyma cells also (see page 605). The merozoites of a rupturing merocyst are a little more differentiated in structure than the nuclei of the mature cyst. They consist of a lighter and a darker staining portion, almost giving the effect of a very young malarial ring. This biphasic staining effect enables them to be easily distinguished from other structures in the liver tissue.

It is, unfortunately, no more possible to distinguish sexual (*i.e.*, blood gametocyte) from asexual (*i.e.*, to develop into a new merocyst) forms in this infection, than it is in the case of merozoites of an ordinary malarial parasite. But for reasons which have already been given (page 603), and also because merozoites can actually be seen within hepatic cells, it is practically certain that some of the escaping parasites must be asexual in nature. I have already described (page 602) the youngest forms seen in the blood in *P. Kochi* infections, and it will be noted how closely they resemble these biphasic forms in the liver.

#### INFECTIVITY OF MEROCYSTS.

It is difficult to understand why merozoites obtained from merocysts fail to grow into gametocytes in culture. A cyst was removed from the liver of an affected monkey, crushed and placed in defibrinated blood from a clean animal with a few drops of 50 per cent. glucose solution. The culture was inoculated at 37° C. and examined with negative results, 2 and 5 days later. In the same way merocysts from three infected animals were excised, ground in normal saline and the suspension was inoculated intraperitoneally and intra-hepatically into three clean monkeys. No infections developed. Lastly on the assumption that recently escaped merozoites should be free in the plasma of a heavily infected monkey the plasma was separated and inoculated into two animals. No gametocytes subsequently appeared in their blood, although three controls inoculated with whole blood developed the usual brief infections. It is possible that all these negative results may have been due to the immaturity of the merocysts used in the experiments. It may be noted that inoculation of organs containing schizonts of *Haemoproteus* also gives poor results. COATNEY (1933) found that one bird only out of four developed a light infection. It appears therefore that the tissue forms of the *Haemoproteidae* are relatively

uninfective on subinoculation as compared with similar (*i.e.*, exoerythrocytic) forms of avian plasmodia

### MEROCYST RUPTURE AND PARASITAEMIA

The blood picture provides in some cases confirmatory evidence of merocyst development and rupture. In most infections the liver foci are few in number and therefore theoretically should produce crops of new gametocytes in the peripheral blood at irregular and sparse intervals. The actual parasitaemia in three such cases is shown below —

TABLE I

MONKEY LL —Number of Parasites in Approximately 8,000 R B C s

Date	Rings	Half-grown forms	Mature gametocytes
1946 Dec. 23	108	46	26
24	48	36	104
25	6	54	72
26	4	16	110
27	2	6	100
28	76	0	64
29	94	4	62
31	2	26	54
(Liver had about 15 visible merocysts)			

TABLE II

MONKEY DD —Number of Parasites per 8,000 R B C s Approximately

Date	Rings	Half-grown forms	Mature gametocytes
1946 Dec. 1	20	2	57
2	24	8	53
3	53	7	61
4	37	7	87
5	22	9	54
6	24	7	56
7	40	3	91
8	31	9	76



TABLE III.  
MONKEY KIK.—PARASITAEMIA.

Date.			Rings.	Half-grown forms.	Mature gametocytes.
1947	Jan.	2	5 in 50 fields	3 in 50 fields	20 in 50 fields
		4	Few	numerous	numerous
		5		Few	
		6			
		7	None		
		8	Few	Nd	
		9			
		10			
		11		Few	
		12	None	Nd	
		14	Few	Few	
		15	Numerous	Nd	
		16	Less numerous	Few	
	Feb.	9	Numerous		
		7		Nd	
		8	Few	Numerous	
		9	None	Few	
		10	Numerous		
		11	Few	Nd	
		12		Numerous	
		13			
		14			

(Liver had about 20 viable merozoites.)

Monkey L.L. showed clearly a sudden flooding of the circulation with young rings on the 23.12.46 and again on the 28.12.46. An increase in ring forms at irregular intervals is also to be noted in the other two monkeys.

In the rarer cases, when the liver contains numerous foci, merozoites must be discharged nearly continuously and the blood picture should show no such fluctuations in the relative parasitaemia. Monkey R.R. illustrates this point.

TABLE IV  
MONKEY R.R.—PARASITAEMIA.

Date.		Rings.	Half-grown forms	Mature gametocytes.
1947	Feb. 23	24	4	26
	24	24	5	1
	25	26	4	20
	26	27	7	16
	27	28	9	19

The absence of synchronicity in *P. kochi* is in contrast to ordinary malaria infections with their well-defined periodicities. Certain observers have reported various "cycles" for *P. kochi*, but there has been no uniformity in their findings, and this is easily understood now that the nature of schizogony has been elucidated.

### TISSUE REACTIONS

The tissue phase of development of *P. kochi* is accompanied by highly characteristic cellular reactions which present an interesting pathological study. The changes that take place in the parasitized hepatic cell have already been described. Briefly, there is an enormous hypertrophy of this cell, with repeated nuclear division. The reaction that occurs around the cell and later around the mature merocyst will now be described. There is no tissue response whatsoever around the growing parasite, and it is not until fluid begins to accumulate in the merocyst that phagocytes appear. Even forms nearly  $150\mu$  in diameter lie undisturbed in between the columns of liver cells which are merely pushed aside.

The attack apparently commences when the parasite is undergoing differentiation into cytomeres. Large numbers of polymorphonuclear leucocytes accumulate and break their way in between the cytomeres which are thereby rendered more conspicuous. The process may continue until all trace of the parasite disappears in a small abscess. More commonly the onslaught is less successful and the thick wavy border of the parasite resists penetration, most of the polymorphs disappearing or remaining in small groups around the merocyst, particularly adjacent to that part which is in a slightly younger stage of development.

The next attack seems to come from cells of the lymphoid macrophage system. Lymphocytes, polyblasts, macrophages and fibroblasts become concentrated between the parasite and the liver parenchyma. Until the merocyst ruptures, these cells apparently make little attempt to invade the parasite, they are always outside the hyaline border, though they frequently occupy the hollows in its wavy edge. At about the same time, two very characteristic features appear, giant cells and cells containing bright eosinophil granules. The giant cells are in close proximity to the parasite. They may be more of the nature of a syncytium with an ill-defined edge and irregular processes, or they may resolve into discreet oval cells of large size, and sometimes such forms unite to form a single cell of striking appearance (Fig 11). The function of these giant cells is apparently phagocytic for red granular (? merozoite) debris is found within them. Their origin is probably the polyblast (MAXIMOW and

BLOOM, 1938) it is unlikely that they represent the final result of the multiplication of the nuclei of the original liver cell for if that were so they would presumably be still within the hyaline border (i.e., remains of cell membrane) instead of exterior to it.

The cells containing eosinophil granules have been called eosinophiles by both LEVADITI and SCHOEN (1932) and by HAWKING and HUNT (1947). They are, however apparently not eosinophil leucocytes, but phagocytic monocytes, the granules of which, in some instances at least, are the merozoites of the parasite. These cells can also be recognized in smears, and the red granules then sometimes show the biphasic staining characteristic of the mature merozoites. The nucleus of the cell is a large oval body the granules may be confined to a small portion of the protoplasm in a discreet group surrounded by a halo.

The next and final stage is the invasion of the ruptured merocyst and its rapid destruction by the phagocytes. The last part of the parasite to resist is the hyaline border and this can occasionally be recognized in the centre of a focal lesion, consisting of giant cells, fibroblasts, polyblasts and lymphocytes, no longer in special layers. Focal infiltrations may be seen in the livers of uninfected monkeys, but are easily differentiated by the absence of the giant cells and of the cells containing eosinophil granules.

Scars of old foci may be seen on the surface of the livers and in nearly every infection there is a mixture of resolving opaque merocysts and of mature transparent ones. In infections which are dying out the liver shows only small fibrotic spots, which on section show the typical cellular reaction around perhaps a dark mass representing all that is left of the parasite (Fig. 12).

## DISCUSSION

The evidence that the liver forms are part of the *P. kochi* cycle was presented in the earlier paper. Further examples in positive and negative animals have confirmed this and the discovery of all stages in the development of the parasite has provided a picture of the complete cycle in the monkey.

It is now clear that *kochi* does not belong to the Plasmodiidae, which is defined as a family producing forms which live in the red blood cells during the asexual cycle.

HAWKING and HUNT (1947) have called *kochi* a *Haemoproteus* but the appearance of the developmental stages in the liver are so unlike the *Haemoproteus* schizonts that in my opinion this is incorrect. *Kochi* develops in the parenchymatous cells of the liver producing a highly characteristic body with multi ple

invaginations of its surface. *Haemoproteus* develops as well-defined cytomeres in the endothelial cells of blood vessels. Then, the final form in *kochi* is the merocyst, a spherical body containing fluid and lined by the remains of the developing parasite consisting of innumerable merozoites. In *Haemoproteus* there is no such cyst-like formation, though the schizonts are grouped in masses separated by septa. Although of little taxonomic significance, it may be noted that *Haemoproteus* has halter-shaped gametocytes in nucleated erythrocytes, whilst *kochi* has spherical gametocytes in non-nucleated erythrocytes.

*Kochi* has closer morphological affinities with *Leucocytozoon*, which, as described by HUFF (1942) goes through a remarkable development in the liver, heart and other organs. The schizonts grow in the parenchyma cells of the liver, the borders and nuclei of which enlarge to an enormous extent. Cytomere formation follows with the eventual production of a packed mass of thousands of merozoites in a megalo-schizont up to  $105\mu$  in diameter. This bursts and discharges the bipolar staining merozoites into the surrounding tissues. There is an obvious parallel here to the *kochi* tissue phase—the major points of difference being the absence of peripheral nuclei in the youngest forms of *Leucocytozoon*, of the convolutions of the half-grown, and of the merocyst formation in the mature.

It appears, then, that *kochi* should be placed in a third genus in the family Haemoproteidae, and the correct generic name would appear to be *Hepatocystes* (LEVADITI and SCHOEN, 1932). The amended genus is defined as follows.

A sporozoan parasite of the lower African monkeys, which develops in the parenchyma cells of the liver, first as a minute round body consisting largely of chromatin, then as a sphere with peripheral nuclei. The nuclei later become scattered throughout the cytoplasm, the surface of which folds into multiple invaginations. Vacuolation occurs and fluid accumulates in a large central vacuole which expands to form the merocyst. The merozoites collect in enormous numbers in the cyst wall, which eventually ruptures and the majority of the merozoites escape into the circulation where they invade red blood cells and grow into spherical male and female pigmented gametocytes.

Genotype *Hepatocystes kochi* (syn *Haemamoeba kochi*, LAVERAN, 1899, *Hepatocystes simuae*, LEVADITI and SCHOEN, 1932, *Haemoproteus kochi*, HAWKING and HUNT, 1947).

Habitat Tropical Africa

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## SCHISTOSOMIASIS IN THE KOTA KOTA DISTRICT OF NYASALAND

BY

O N RANSFORD, M D (LOND ), D A (ENG ) \*

### GENERAL

Schistosomiasis has long been recognized as one of the chief causes of morbidity among the natives of Nyasaland. Urinary schistosomiasis is the dominant form, although in some parts of the Protectorate, the intestinal form is common too. The magnitude of the present problem was recently emphasized by CULLINAN (1945), who notes a urinary schistosomiasis incidence of 41.59 per cent in a group of Nyasaland soldiers.

Medical surveys during the period 1935 to 1937 show that the incidence of both forms of the disease is highest in the low-lying parts of the country.

Schistosomiasis in the northern part of the Protectorate was investigated by DYE (1922-24). He described the clinical syndrome, similar to Egyptian splenomegaly which is due to heavy and repeated infection with *Schistosoma mansoni*. *Planorbis* sp. (near *P. sudanicus*) was identified as the intermediate host of the fluke. DYE considered that *Physopsis globosa*† was the probable host of *S. haematobium*, but certain proof was not obtained. *Melania* (*Melanoides*) *nodicincta* was also suspected as a possible vector. GOPSILL (1931), working in the southern part of the country, found that 80 per cent of 500 consecutive urine examinations showed infection with *S. haematobium*. He concluded that *M. tuberculata* was the probable vector of urinary schistosomiasis in this area.

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†According to the British Museum (Natural History) the accepted name for this snail is now *Bulinus globosus*.

on the Shire River but again certain proof was lacking. A similarly high infectivity rate with urinary schistosomiasis has been reported from other districts where they border on Lake Nyasa.

This paper summarizes the results of observations made in the Kota Kota District of Nyasaland over a 12 month period. This district, with a land area of 2,418 square miles, extends up the western shore of Lake Nyasa for nearly 100 miles and is 45 miles broad at its widest point.

The district supports a population of between 80 000 and 90 000 which is approximately equally divided between the low-lying lake shore country and an undulating plateau lying to the west. These two areas are separated from each other by a broad strip of barren, infertile country of sand and stone which is infested with tsetse fly and, being uninhabited, discourages travel between the two populated areas.

The lake shore strip about 1 600 feet above sea level, is sandy except for fertile alluvial patches lying at the mouths of several large rivers. The people here grow cassava as their staple crop and catch good deal of fish. As a cash crop they grow rice the acreage of which has increased during the last 15 years. Latterly although influenced both by rainfall and demand, between 400 and 600 short tons of rice are grown for sale annually and it is estimated that nearly 10 000 natives work in the paddy fields during the season.

The hill country rises gradually from the lake and in part attains an altitude of over 5 000 feet. It is formed of fertile foothills with rich valleys, sloping up to cold fertile grass uplands, whose soil contains a higher content of mineral salts than that of the lake shore. The hill people grow maize as their staple crop.

Nyasaland is not a country of large native communities, and Kota Kota town, with about 4 000 huts, is considered to be the largest of them in the Protectorate. The town lies on the shore of Lake Nyasa, about half-way up the district. It is about 4 miles long and  $\frac{1}{2}$  mile wide.

Heavy rains (between 40 and 60 inches) fall between November and April. Streams run swiftly down the hills but more slowly as they approach Lake Nyasa. In the near vicinity of the lake, these streams pile up silt, and during the dry season series of shallow pools are left along the stream courses near to the lake.

These pools gradually dry up as the dry season continues. During this time they are used as a water supply and as a refuse receptacle, to be preferred to the crocodile-infested lake. Especially when the vegetation affords some degree of privacy the surroundings of these pools are in frequent use as latrines while towards the end of the dry season, when the surrounding vegetation has been burned, trampled down, or cleared away children are constantly to be seen bathing in them. A considerable proportion of the lake shore population is Mohammedan, whose excretory habits are associated with water.

It will be appreciated that the association of the lake shore people with these dry weather pools is a close one, especially towards the end of the hot season, when the quantity of water is diminished and is most foul. Conditions in fact are ideal for the natural cycle of the human schistosomes.

Infection with *S. haematobium* does not give rise to such sufficiently painful early symptoms as to make the African seek hospital treatment until complications arise. Infection has rarely been a reason for rejection of military recruits. Infection with intestinal schistosomiasis is sometimes more serious. The same clinical syndrome of hepatic cirrhosis and splenomegally reported by DYE is seen in Kota Kota from time to time.

It is the debilitating effects of urinary schistosomiasis, especially as it affects the mental development of African children which makes its control of great importance in the district. It is considered that the undertaking of more efficient control methods will prevent the cumulative increase in the number of dangerous localities which has been reported from Southern Rhodesia. The southern neighbours of Nyasaland, which receive so many emigrant labourers from the country, would also benefit from the improvement which could be anticipated.

### THE FRESHWATER MOLLUSCA OF KOTA KOTA DISTRICT

Eight mollusc species have been recovered from Lake Nyasa and from freshwater pools in the district. I am very grateful to the late Major M. CONNOLLY and Dr W. J. REES of the British Museum, who kindly identified them as follows —

- (a) *Bulinus (Physopsis) globosus* (Morelet)
- (b) *Biomphalaria pfeifferi* (Kraus)
- (c) *Lymnaea caillaudi* Bourguignat
- (d) *Melanoides tuberculata* (Müller)
- (e) *Bulinus (Pyrogophysa) forskalii* (Ehrenberg)
- (f) *Ampullaria* sp. juv.
- (h) *Lanistes ovum* Tröschel
- (i) *Viviparus unicolor* Olivier

*Physopsis globosa* — This seems to represent *Physopsis africana* north of South Africa. It has been identified as the intermediate host of *S. haematobium* in Southern Rhodesia, Sierra Leone, and Tanganyika Territory and, as will be seen, it is the host in Kota Kota district.

These snails were found in great numbers during the dry season in those residual pools near Lake Nyasa which are close to human habitation. Only very rarely could they be found in similar pools, in uninhabited areas. They were also numerous in village shallow wells. These snails were found, too, in paddy fields during the rains. The snail appears to be a true messmate of man and requires fouling of its habitat for its existence.

During the 12 months in which they were observed they were found to be parasitized by trematodes only between early July and the beginning of the rains.

*Physopsis globosa* was found to be present in only three hill localities. Its occurrence there coincides with a high human infectivity rate with schistosomiasis.

(b) *Biomphalaria pfeifferi* — When these snails were found, they were associated with *P. globosa*, compared with which they are not numerous. These snails are the probable hosts of *S. mansoni* in the district.

(c) *Lymnaea caillaudi* — Although usually associated with *P. globosa*, these snails may occur alone in enormous numbers far from human habitation and, unlike *Physopsis*, can live in fast-flowing water. An occasional snail showed monofurcous styletted cercariae.



(d) *Melanosoides tuberculata*.—These snails are to be found in great numbers during the dry season on the shores of Lake Nyasa. They were infested with a variety of trematode larvae, none of which resembled *S. haematobium*. It is interesting to note that double infections with different trematodes was by no means rare.

(e) *Bulinus forskalii*.—These snails were found in very large numbers throughout the district during the rains, especially in the marshes and rice fields near the lake. They could be seen crawling upstream from the lake to these localities. Despite many dissections, no trematode parasitization was seen, but the possibility of their acting as emergency hosts for schistosome flukes must be borne in mind.

The other three species of snails were found near the lake. They are not known to be of medical importance.

### THE INCIDENCE OF SCHISTOSOMIASIS IN KOTA KOTA DISTRICT

During the period 1935 to 1944 records show that 9,661 urine and 13,000 stool examinations were made on patients attending Kota Kota African hospital. In the urine examinations, 53 per cent. (5189) contained *S. haematobium* ova, this percentage showing little variation from year to year. With the stool examinations, 7 per cent. contained eggs of *S. mansoni* in 1935 the percentage then declines in successive years to under 1 per cent. in 1944. It is evident that in the district, probably because of improvements in sanitation, intestinal schistosomiasis is a disease which is declining in importance, whereas the incidence of urinary schistosomiasis has not shown the same trend.

It was considered that the most accurate method of obtaining more detailed information as to the local incidence of urinary schistosomiasis in the district would be by search for the mollusc vector of the disease, and by village examination of children between 6 and 12 years of age. Children of this age are less likely to have travelled far from their villages than are adults, and probably like experimental animals, they are more easily infected than adults.

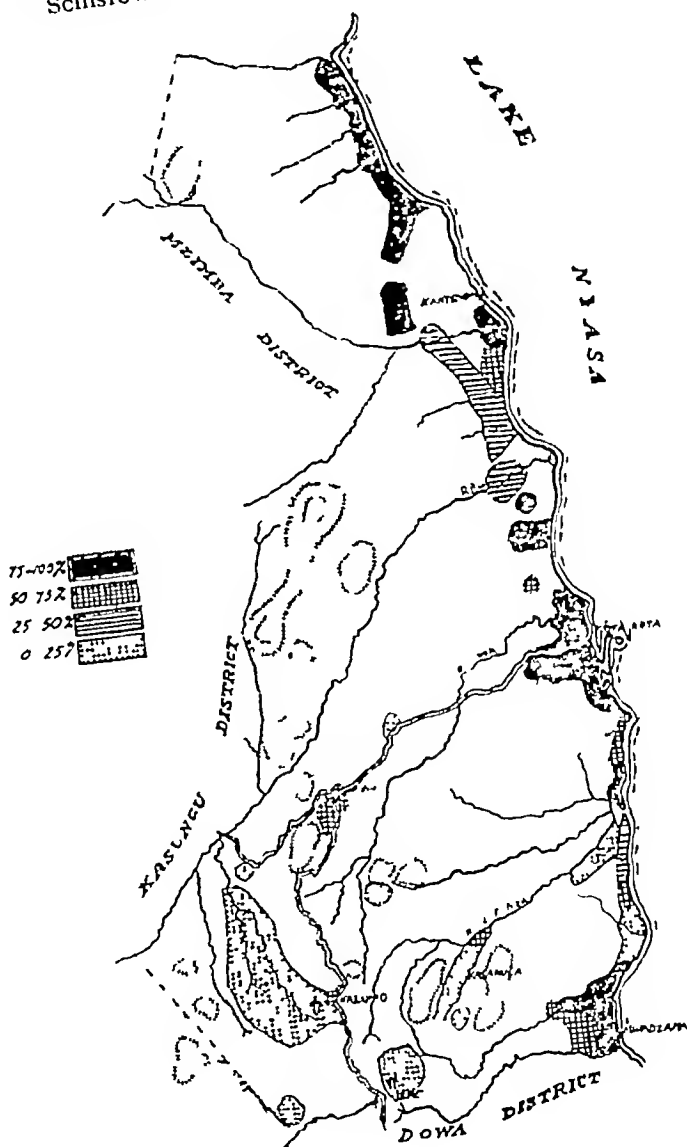
Accordingly the urines of 1,857 children throughout the district were examined, 946 (51 per cent.) of which were found to contain *Schistosoma* ova. This figure represents the absolute minimal incidence. Subsequent examination, especially after a provocative dose of suramin, sometimes revealed ova in children previously reported negative.

Two incidental points are worthy of notice. Apparently boys are more liable to infection than are girls. Thus in 1,519 consecutive examinations, of which 1,000 were on boys and 514 on girls, the infectivity rate of the boys was 58 per cent. and of the girls 49 per cent. Secondly when the urines of smaller children were examined, in no case was a child younger than 4 years found to be infected.

The accompanying map shows how the incidence of urinary schistosomiasis

O N RANSFORD

# SKETCH MAP OF KOTA-KOTA DISTRICT TO SHOW THE INCIDENCE OF URINARY SCHISTOSOMIASIS IN POPULATED AREAS





varies in the different parts of the district. It will be seen that the greatest incidence is along the shores of Lake Nyasa and that the incidence decreases with altitude. In Kota Kota town, 81 per cent of the children were infected. Only three foci of heavy infection were noted in the hill areas.

In all cases, a high village incidence of schistosomiasis was found to be associated with the presence of *P. globosa* in pools or wells nearby. Their presence there may be limited to only a few weeks of the year. No similar relationship with any other mollusc species was found.

The absence of *P. globosa* from all but a few localities in the hills is due to the fact that the village source of water is usually from fast-flowing streams. That it is not due to climatic conditions is shown by the facts that snails exist in the three foci mentioned, and that snails were kept alive experimentally at a high altitude. Cold by discouraging bathing may, however, be a factor, in hill country, which limits infection despite the presence of a mollusc vector of the disease.

The use of such a survey is to indicate where control can be most advantageously applied. In this district, lake shore control could be applied first to a heavily infested locality, Kota Kota town, and from there be gradually extended to include the whole area. At the same time control could be applied to the isolated hill foci where success is likely to be comparatively easily obtained.

#### THE INTERMEDIATE HOST OF *Schistosoma haematobium* IN THE DISTRICT

Of the different snails found in the district, only one—*P. globosa*—was always associated with foci of urinary schistosomiasis, and in 1,200 dissections of possible snail hosts only this snail was found to harbour furcocercous cercariae resembling *S. haematobium*. Experimental proof that *P. globosa* is a host was obtained in the laboratory, and I am indebted to Dr DE MEILLON for his advice on the method adopted. Having bred "clean" *P. globosa* in the laboratory, they were each exposed to five miracidiae obtained from *S. haematobium* eggs from freshly voided urine, after they had been repeatedly washed in normal saline. Cercariae of *S. haematobium* were obtained from one snail 8 weeks after exposure. Exposure to more than five miracidiae caused death of the snails in a few days or weeks. It was not possible in an out-station to make any experimental infestations of laboratory animals.

Other molluscs, notably *B. forskalii* and *Lymnaea caillaudi*, may act as emergency hosts for human schistosomes, but from study of their distribution and seasonal incidence in the district, it is considered that this can only be a rare occurrence.

It is important to realize that man may not be the only vertebrate host of human schistosomiasis in nature. The possible role of small rodents, goats, etc., acting as reservoir hosts, should be borne in mind. Many workers have reported successful experimental infection of laboratory animals with the

cercariae of human schistosomes. Thus ANNIE PORTER describes a series of experiments in which laboratory bred white rats, puppies and guineapigs were successfully infected with schistosome cercariae obtained from four South Africa mollusca. Certainly the related *S. japonicum* uses domestic animals and field mice as alternative hosts. Probably the fluke of urinary schistosomiasis is not so limited in its range of definitive host as it was formerly considered to be. In a limited series of examinations, no evidence of the existence of a reservoir host in Kota Kota was obtained.

### THE CONTROL OF SCHISTOSOMIASIS IN THE DISTRICT

The control of schistosomiasis in the district really lies in the hands of the sanitary engineer. Much may be accomplished by education, by the encouragement of natural enemies of snails, by the use of mollusc poisons, and possibly by attempting the mass treatment of Africans. But the only hope of permanent control will be obtained by the elimination of the village pools lying close to Lake Nyasa, whether it be by drainage, filling in, or by the diversion and utilization of the water in deep wells. The emphasis of a control scheme, as elsewhere in the world, must be laid on snail destruction and on the prevention of their present close association with man. The most difficult single problem is to deal with the lake lagoons and backwaters and the lake water where the shore is overgrown with reeds. Fortunately such sites are not usually important foci of infection. The water of the Lake Nyasa proper is safe.

Nature, by its alternate flushing with rain and drying, maintains a somewhat precariously balanced population of the mollusc vectors of human urinary schistosomiasis. Bad sanitation will tip the balance in the snail's favour and the converse is equally true. Indeed, it is possible that the increasing use by Africans of deep pit latrines over the last decade has played its part in reducing the numbers of the snail vector of intestinal schistosomiasis.

Control of schistosomiasis is always a local problem. This is well illustrated in Kota Kota town. Here a supply of safe water could be made available to a considerable portion of the population, merely by piping water from the hot springs lying less than a mile from the town.

Eventually it may become possible to deal with every semi-stagnant pool lying near a village or major route of African travel. Each would have to be considered on its own merits. In Kota Kota town, where a large population is affected, drainage into the lake is the best means of elimination of many pools. Meanwhile, temporary control can best be achieved by the use of naturally occurring snail poisons.

MOXLEY (1938, 1944) has reported on the use of natural poisons in Tanganyika and in Southern Rhodesia. Some investigations of poisons occurring in Kota Kota were carried out.

For natural molluscicides to be of use, they must fulfil three conditions

- (a) They should be lethal to snails in practical concentrations
- (b) They should be harmless to man in the same concentrations
- (c) They should be easily and cheaply grown in quantities adequate for the snail localities concerned

In the lake shore areas of Nyasaland various naturally occurring fish poisons are well known and used by the natives. These poisons also kill frogs, tadpoles and snails. In this respect the poisons may be judged to have "passed" their field trials in native hands.

It was found that a large variety of plants were lethal to snails in the laboratory, but after field trials only three plants were considered to be of practical use. Laboratory experiments are, however, necessary in order to obtain quantitative information as to the lethal qualities of the poison.

For the purpose freshly collected specimens of adult *P. globosa* measuring between 11 mm and 15 mm in length, were used. Younger snails were found to be too susceptible to the poisons. Each experiment was conducted over a period of 48 hours using two controls, one of fresh water and one of copper sulphate 1/400,000, which is lethal to snails within that period. The criterion of death of the snails was failure to revive after being placed in clean water for 24 hours.

The three poisons found to fulfil condition (a) above, and their minimum lethal concentrations, were approximately —

*Tephrosia vogeli* leaves, 1/4,000

*Swartzia madagascariensis*, 1/3,000

*Mucuna* sp ("Dema"), 1/1,500

Of these only *Tephrosia vogeli* (which contains tephrosin) fulfilled condition (c). Its use as a molluscicide was suggested by MOZLEY, who found that it had no injurious effects when ingested by mammals. Although Africans will not drink for some days from pools in which it has been used as a fish poison, volunteers who drank 1/2 pint of a 1/2,000 extract suffered no ill effects.

*Tephrosia* can be cultivated easily and cheaply on the lake shore, it is available in much larger quantities than either *Swartzia* or *Mucuna*, and is recognized by the natives as the more efficient fish poison. Sufficient leaves to treat 500 gallons of water can at present be bought for one penny.

A record of an experiment—one of many—is given below. It was conducted to determine the minimum lethal concentration of *Tephrosia* leaves.

Hand crushed leaves of *Tephrosia vogeli* were added to vessels containing 10 litres of fresh spring water in each of which twenty *Bulinus* (*Physopsis*) *globosa* had been living for 24 hours. Controls of copper sulphate and water were used.

Kill	<i>Tephrosia</i> leaves.				CuSO <sub>4</sub> 1 400 ppm	Spring water
	1 000	1 400	1 600	1 1000		
4 hour	18	9	1	0	18	8
48 hour	20	20	16	1	20	0

Other experiments showed that hand-torn leaves are almost as effective as *Tephrosia* leaves pounded in the mortar and that dry leaves are as effective as the fresh leaves.

The method used in conducting field trials on this and other plants was as follows: a suitable pool was selected and early on successive mornings the snails in the pool were counted and returned. The search lasted for the same time each day and varied from 15 minutes to 1 hour according to the size of the pool. When the count on 3 successive days was constant or nearly constant, the poison under test was added to the pool and counts of live and dead snails made on the next 2 mornings.

As weather conditions affect the ease with which physopne snails are found, it is important to conduct counts at the same hour each day and before the water has been disturbed.

In the case of *Tephrosia* the leaves were wetted, and torn up by hand, the green juice then being distributed uniformly through the pond. Satisfactory counts of dead snails were obtained when *Tephrosia vogelii* was tested, the field trials confirming its efficiency as a snail poison.

Similar experiments were conducted with malachite obtained from Southern Rhodesia. The sample used was not so effective as tephrosia leaves, and the high cost of importing malachite renders its local use uneconomic.

For an extensive control scheme based on the use of *T. vogelii*, the plant should be cultivated close to the pools in which its use is intended. Its application would most profitably be confined to the period between July and September when the snails are easily accessible and before the majority become infective. Especially when there is mud and silt in the pools, a concentration of 1 2,000 or of 1 3,000 should be aimed at rather than the minimum lethal concentration. It was not in practice easy to judge the volumes of the larger pools with any exactitude, but the African assistants who worked in the field experiments were soon able to estimate the necessary quantity of leaves.

Any measure for the control of schistosomiasis which depends upon the use of snail poisons carries the disadvantage entailed in the necessity of yearly repetition. It is felt, however that until such time as the permanent control of schistosomiasis is effected by the improvement of urban and rural sanitation in Kota Kota, some scheme based upon the use of *T. vogelii* should be carried out. It offers hope of reversing locally the present trend towards increase in

the number of dangerous snail localities, which exists in Central Africa today. The advantages which would accrue from a policy which tips the balance against the snail vectors of schistosomiasis would be felt far beyond the district boundaries.

#### A NOTE ON *Tephrosia vogeli*

*Tephrosia vogeli* Hook. f. is a perennial shrub reaching a height of 8 or 9 feet. Although the flowers are usually white, red and purple varieties occur in some parts of the world. The leaves are long and narrow, varying in length from 1 to 3 inches, in breadth from  $\frac{1}{2}$  to  $\frac{3}{4}$  inch, of yellowish green colour, and with a somewhat pungent odour. The stems are hairy, as are the pods. The latter contain a large number of brown kidney-shaped seeds which have a white hilum. The plant grows equally well at sea level or at 6,000 feet and is easily cultivated.

The plant is cultivated throughout Africa, either casually or by riverine people for its use as a fish poison. Usually the leaves are used separately for this purpose, but the pods are recognized to be more effective and are sometimes used alone or with the leaves. The common practice is to pound the leaves to a pulp to which is added sand or the flour of some cereal, the mixture being applied as uniformly as possible to the water. The dry leaves are equally efficacious.

HANRIOT (1907) examined specimens from the Comoro Islands, and found that the leaves contained a poisonous principle, tephrosin, a crystalline substance, melting at  $187^{\circ}\text{C}$ , which is practically insoluble in cold water. The leaves also contained a volatile oil, tephrosol, and an unclassified yellow substance. Delayed fatal effects on fish were demonstrated with concentrations of tephrosin as low as 1 in 50 million. In higher concentration, the fish turned over and died within an hour.

These results were largely confirmed by workers at the Imperial Institute (1915), who analysed *T. vogeli* from Rhodesia. No evidence of contained alkaloidal or glucosidal substance was found. In their specimens the following percentage quantities were identified: Tephrosin, 0.15 per cent crystals, melting at  $192^{\circ}\text{C}$ ; Tephrosol, 0.06 per cent, a thick yellow volatile oil; Yellow substance, 0.05 per cent, crystals melting at  $228$  to  $229^{\circ}\text{C}$ . The seeds yielded twice as much tephrosin as the leaves, they contained the unknown yellow substance (M.P.  $228$  to  $229^{\circ}\text{C}$ ), and also a new yellow substance melting at  $158^{\circ}\text{C}$ , was isolated.

The possible public health uses of the fish poison have been investigated by a number of workers since the publication of these reports. The value of *Tephrosia* as a mosquito larvicide was noted by ALDERS (1916) who found that the emulsion killed the larvae of *S. fasciata* within 12 hours. CAYSTON (1928) suggested its application as a snail poison in *Bilharzia* control. In 1934 WORSLEY confirmed its value against mosquito larvae while in the same year WILLIAMS reported verification of its molluscicidal properties. The insecticidal properties of *T. vogeli* were studied by PATTERSFIELD *et al* (1928, 1937) and they noted its strong repellent action to certain biting insect. HOLMAN (1940) reported its effective parasiticidal action when used by natives against fleas, lice and ticks. The closely related substance, rotenone, is an effective sarcopticicide, and in 1942, working in Nyasaland, W. F. C. BROWN reported that *T. vogeli* had similar properties. MOZLEY (1944) included *T. vogeli* in his investigations of the value of poisonous plants in Southern Rhodesia as snail poisons. Despite these investigations by numerous workers there seems to have been little or no use made of the insecticidal or molluscicidal properties of the plant either in the fields of agriculture or of preventive medicine.

Reports on the effect of *T. vogeli* on animals are not numerous and they are somewhat contradictory. DUGGILL (1937) pointed out that persons wading in polluted water to draw or to catch fish suffer the complaint of a numbness of the legs or of a roughness of the skin. While considered to be thus harmful to man in one locality the same writer elsewhere noted that the leaves are used for their antiseptic properties in dressing for sores. HANRIOT (1907) found that when given orally, in doses as high as 1 gramme, teph-



roots had no effect on dogs, and he noted that rabbits eat the leaves with impunity. When given by hypodermic injection, however, in doses of 0.1 grammes/kg., fatal convulsions and paralysis was produced in both dogs and rabbits. In his paper on the insecticidal properties of *T. rogersi*, WORMLEY states that "tephrosin is non-poisonous to human beings and animals." The present writer was unable to confirm the presence of parasitæ after wading in poisoned water nor were any toxic signs noted in the course of treatment with tephrosin emulsion of many cases of scabies. No ill effects were suffered by four volunteers who drank  $\frac{1}{2}$  pint of 1:2,000 extract. The conclusion was reached that no harmful effects are to be expected in man, provided that such concentration is not exceeded in the field.

## SUMMARY

1 The results are given of a snail survey in the Kots Kots district of Nyasaland.

2 The results of an investigation into the local incidence of urinary schistosomiasis in the district are noted.

3 The snail *Balanus (Phytopus) globosa* (Morelet) is incriminated as the intermediate host of schistosomiasis in the area.

4 Control methods are discussed. Until such time as rural sanitation becomes more developed, the use of the easily cultivated fish poison—*Tephrosia rogersi*—as a molluscicide is advocated.

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# COMPARATIVE MERITS OF STERNUM, SPLEEN AND LIVER PUNCTURES IN THE STUDY OF HUMAN VISCERAL LEISHMANIASIS

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For the diagnosis of kala-azar the most common methods nowadays employed are sternum, spleen and liver punctures. The preference of one method to another varies in the hands of each clinician and the diagnostic value of each is by no means definitely determined and agreed upon by them. A study of their relative merits in detecting leishmania from patients prior to, during and after antimony therapy, has therefore been undertaken by us.

## METHOD OF STUDY

Patients suspected of visceral leishmaniasis are each examined by sternum and spleen punctures. Occasionally liver puncture is added. Being thus diagnosed, the patient is then treated with a course of urea-stibamine, the

total dose of which is 0.02 to 0.035 gramme per kg. of bodyweight to be evenly divided into six weekly or bi weekly doses for intravenous injection. During the period of treatment as well as subsequently the patient is examined periodically for persistence of leishmanial parasites in bone marrow spleen and liver.

The patient to be examined is laid on his back on an examination table. The skin over the spot chosen for the puncture is prepared with 2.5 per cent. tinct. iodine and 70 per cent. alcohol and the operation performed under strictly sterile technique. Thin smears are prepared from tissues obtained by successful punctures, stained (Wright's stain) and examined for leishmania.

### TECHNIQUE

The method used is briefly described below

#### (1) STERNUM PUNCTURE

The needle used is shortened lumbar puncture needle or hypodermic needle of 18-20 gauge with stylet attached. The sternum is entered at the level of the second intercostal space. After piercing through the skin and subcutaneous tissues, the needle, pointing either anteriorly or posteriorly (at an angle of 45 to 80° with the skin, is pushed with moderate force by drilling movement through the anterior cortex of the sternum until the marrow cavity is reached. When the marrow cavity is reached, which is indicated by sudden yielding of resistance to the drilling needle the stylet is removed and 10 c.c. or 20 c.c. syringe attached to the needle. The marrow is aspirated with short jerking movement until it appears just at the proximal end of the needle. This can be accomplished by disconnecting the syringe from the needle after one or two aspirations, and observing whether the marrow fluid has appeared or not. It looks bloody and oily containing whitish tissue-particles.

#### (2) SPLEEN PUNCTURE

The size of the spleen is first determined by palpation. An ordinary 20-gauge hypodermic needle attached to 20 c.c. syringe is inserted just under the costal margin along the mid-longitudinal line of the enlarged organ or slightly upward at one of the intercostal spaces, when the organ is barely palpable. The operation consists of two movements: the first, the piercing of skin, subcutaneous tissue and muscle is done with much deliberation; the second, puncture of the spleen and suction with the syringe during the rapid withdrawal of the needle is to be accomplished within 1½ seconds. During the second tempo the patient is told either to hold his breath or puncture is made during expiration in case of crying child, with the needle pointing anteriorly at an angle of 45 to 60° with the skin at a horizontal plane. A successful puncture will yield only tiny amount of splenic tissue inside the needle and no blood should appear in the syringe.

#### (3) LIVER PUNCTURE

The needle used is the same as that used in spleen puncture. The needle enters the sixth or seventh intercostal space along the right anterior axillary line over the hepatic dullness. After piercing through skin and subcutaneous tissue the needle is pushed through muscle along the upper border of the rib. The liver is then punctured quickly and suction is made with the attached syringe during rapid withdrawal of the needle. Like the spleen puncture, successful puncture will yield only tiny bit of hepatic tissue inside the needle.

## EXPERIMENTS AND RESULTS

(A) DETECTION OF *Leishmania* BY STERNUM AND SPLEEN PUNCTURES

1 - *Prior to Antimony Therapy*—Sternum and spleen punctures were done in parallel in a series of 450 subsequently proven cases of kala-azar, and it was found that the former method was positive in 386 (85.8 per cent) and the latter in all but ten (97.6 per cent) cases. Three hundred and seventy-five (83.3 per cent) patients revealed leishmanial parasites by both methods, and the remaining seventy-five by either one of the two punctures: sixty-four (14.2 per cent) by spleen puncture only and eleven (2.4 per cent) by sternum puncture only (Table I). Of the 375 cases, in which the parasites were found in both bone marrow and spleen, 184 (49.1 per cent) showed more parasites

TABLE I  
DETECTION OF LEISHMANIA IN 450 CASES OF KALA-AZAR BY  
STERNUM AND SPLEEN PUNCTURE

Number of patients examined	Results					
	Both punctures positive		Only sternum punc- ture positive		Only spleen punc- ture positive	
	Number	%	Number	%	Number	%
450	375	83.4	11	2.4	64	14.2

and thirty-four (9.1 per cent) less parasites in the spleen puncture smear than the sternum puncture smear, and the remaining 157 (41.8 per cent) revealed an approximately equal number of parasites by both methods (Table II). The bone marrow smears of the eleven patients, in whom only sternum puncture was positive, were each found to have only a few parasites, while many of the spleen smears prepared from the sixty-four patients, in whom only spleen puncture was positive, showed a large number. It is evident that spleen puncture detects more positive cases of kala-azar than sternum puncture and that positive smears prepared by the former method often revealed more parasites than those prepared by the latter one.

2 - *During Antimony Therapy*—Two hundred and three cases were under study. It was found that in 68 per cent leishmania disappeared from the bone marrow and spleen at about the same time after the same number of injections, while in the remaining 32 per cent there was some discrepancy in time for the disappearance of the parasites from these two organs (Table III). However, in a large majority of cases, once one of the two organs was cleared of leishmanial parasites, the other would invariably be rendered sterile after

TABLE II.

RELATIVE NUMBER OF LEISHMANIA IN 375 PAIRS OF STERNUM AND  
SPLEEN PUNCTURE SMEARS PREPARED FROM 275 PATIENTS.

Sternum puncture.	Spleen puncture	Number of cases.
++++	+	9
+++	+++ or ++	19
+++ or ++	+	10
+++	++	9
		<hr/> 34 or 9.1 per cent.
+	++++	19
+	+++ or ++	85
++ or ++	++++	74
++	+++	36
		<hr/> 184 or 49.1 per cent.
+	+	30
+++ or ++	+++ or ++	70
+++	+++	51
		<hr/> 197 or 41.9 per cent.

Explanation of markings

++++ indicates more than fifty leishmanias per ten microscopic fields.

+++ indicates ten to fifty leishmanias per ten microscopic fields.

++ indicates one to nine leishmanias per ten microscopic fields.

+ indicates less than one leishmania per ten microscopic fields.

(Magnification 450)

one or two more injections. There were about 5 per cent. of cases in which the spleen was no longer palpable when the sternum puncture remained positive or first became negative for leishmania.

† In another series of twenty-eight cases, in which leishmanial parasites were detected prior to therapy by only one method, either sternum or spleen puncture, it was observed that the parasites in these cases disappeared under treatment very rapidly in most cases after one or two injections and in none did the parasites persist after the fourth injection. Flare-up of parasites in the bone marrow of a patient originally with a negative sternum puncture after one or two injections was also noticed in a few instances, but the parasites from such a patient would usually be eradicated after the fourth injection.

3. *After Completion of Antimony Therapy*—In a follow-up of treated cases, sternum and spleen puncture were found to check up each other very closely. It was observed that whenever the spleen became non-palpable, the sternum puncture was always negative for leishmania. In symptom-free

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TABLE III

RATE OF DISAPPEARANCE OF LEISHMANIA FROM BONE MARROW AND SPLEEN OF 203 PATIENTS UNDER UREA-STIBAMINT

Time of exam in relation to number of injection (after)	Number of patients examined for first time	Number of re-exams for patients with positive spleen and sternum punctures during treatment	Results		
			Sternum puncture Spleen puncture	Neg Neg	Pos Neg Pos
First	27	0		6	6
Second	66	3		31	8
Third	64	10		46	14
Fourth	22	16		13	5
Fifth	3	4		4	4
Sixth	3	7		24	1
Seventh or more	21	5		6	0
	0			4	4
				21	0
				4	0
				138	41
				(68.0%)	(20.2%)
					21
					(11.8%)
Total	203	45			

treated patients with palpable spleen, not only would sternum puncture be negative, but also spleen puncture would fail to reveal leishmanial parasites in the still enlarged organ. Thirty-two such cases were recorded by us.

(B) DETECTION OF *Leishmania* BY LIVER PUNCTURE

In another series of 121 cases (included in the 450 cases mentioned above), liver puncture was added at the same time. It was found that spleen puncture was positive in 118 (97.6 per cent) cases, sternum puncture in 108 (89.2 per cent), and liver puncture in 93 (76.9 per cent). Nine cases showed leishmanial parasites by spleen puncture only, one case by sternum puncture only but none by liver puncture only (Table IV).

Under urea-stibamine therapy the parasites disappeared much sooner from the liver than either spleen or bone marrow, as determined by means of needle biopsy. Of eighty-six cases under study, forty-five were found to have the parasites disappear from these three organs at about the same time after the same number of injections, while in the remaining forty-one the parasites always disappeared from the liver first, although in some of them from either the bone marrow or the spleen too (Table V).

TABLE IV

DETECTION OF LEISHMANIA IN 11 CASES OF KALA-AJAR BY STERNUM, SPLEEN AND LIVER PUNCTURES.

Patients examined.		Results of punctures.		
Number	Per cent.	Sternum.	Spleen.	Liver
07	71.0	Pos.	Pos.	Pos.
10	14.0	Pos.	Pos.	Neg.
0	7.4	Neg.	Pos.	Neg.
4	3.2	Neg.	Pos.	Pos.
2	1.6	Pos.	Neg.	Pos.
1	0.9	Pos.	Neg.	Neg.
0	0.0	Neg.	Neg.	Pos.

TABLE V

RATE OF DISAPPEARANCE OF LEISHMANIA FROM LIVER, SPLEEN AND BONE MARROW OF EIGHTY-SIX PATIENTS UNDER ORAL-ANTHRAXINE THERAPY

Time of exam. in relation to number of injection (after).	Number of patient examined for first time	Number of re-exams for patients with pos reaction liver and spleen puncture during treatment	Results				
			Liver puncture	Neg.	Neg.	Neg.	Neg.
			Spleen puncture	Neg.	Pos.	Neg.	Pos.
			Sternum puncture	Neg.	Neg.	Pos.	Pos.
First	14	0		4	0	0	1
Second	35	1		15	4	0	2
Third	31	2		16	2	6	6
Fourth	6	4		7	1	0	
Fifth	0	1		0		0	1
Sixth		1				1	
Total	86	8		43	13	13	16
				(50.2%)	(15.1%)	(15.1%)	(17.3%)

## DISCUSSION

A few selected patients in whom a spleen puncture was done because of a negative sternum puncture, and whose spleen subsequently revealed leishmania and *vice versa*, are mentioned from time to time in medical literature. The largest series was the eighty proven cases of kala-azar reported by NAPIER,\* who found the sternum puncture positive for leishmania in seventy-one cases, and who found three positive spleen punctures in six of nine patients in whom no parasites were revealed by sternum puncture. It must be noted here that although these findings gave us a fairly accurate impression of both methods as regards their reliability in detecting leishmania, these experiments were not conducted on a comparable basis. It is apparent that if the relative merits of sternum, spleen and liver punctures are to be evaluated, the puncture of these organs must be performed in parallel, at the same time and in the same case, regardless of the results subsequently shown by each method—as was done in our series of experiments.

Basing the findings of our series of experiments conducted on a sufficiently large number of cases, it was observed that in the diagnosis of kala-azar, spleen puncture would detect more positive cases than either sternum or liver puncture and that the positive spleen puncture smears often showed more parasites than liver or sternum puncture smears obtained from the same case. Of all our cases examined, about 15 per cent revealed leishmania by spleen puncture only, about 2 per cent by sternum puncture only, but none by liver puncture only.

Under antimony therapy, leishmanial parasites were observed to disappear much sooner from liver than either bone marrow or spleen. When the original number of parasites prior to treatment is taken into consideration, it appears that the parasites disappear more readily from the spleen than from bone marrow as determined by means of needle biopsy. Thirty-two per cent of our cases showed some discrepancy in time for the disappearance of parasites from these two organs. It is evident that spleen and sternum puncture should be used in combination in patients, since neither one can replace the other entirely as a means of diagnosis or as a means for ascertaining the persistence of parasites in patients under treatment. While spleen puncture can detect more positive cases of kala-azar, sternum puncture provides a better basis for prognosis since the rate of disappearance of leishmania from a patient under treatment depends more upon the number of parasites originally present in bone marrow than in spleen†, and in ascertaining their final eradication, especially when the spleen is no longer palpable. Liver puncture possesses neither one of these merits and, therefore, should not be encouraged.

Once the technique is mastered, spleen puncture is the simplest of the

\* NAPIER, L. E. (1943) *The Principles and Practice of Tropical Medicine*, p 161  
New York: The Macmillan Company

† Ho, E. A., and his co-workers. Unpublished data on determination of optimum dosage of urea-stibamine for treatment of human visceral leishmaniasis



three, but it is not so certain as sternum puncture, especially since the latter can be repeatedly tried without risk at one session. We fully agreed with NAPIER that, although spleen puncture should not be performed carelessly its dangers are much exaggerated. Except in very anaemic patients in whom the bleeding time was determined, no special precaution was taken by us in preventing post-operative bleeding. So far no accident or complication following spleen puncture has been encountered in our series of cases. It is our impression that if the second movement of the spleen puncture can be performed properly and quickly within  $1\frac{1}{2}$  seconds, the danger of tearing the organ, which may cause fatal bleeding, will be greatly diminished.

### SUMMARY

The relative merits of sternum, spleen and liver puncture in detecting leishmania from kala-azar patients, both before and after urea-stibamine therapy are discussed, and the technique of each method is briefly described.

In a series of 450 proven cases of kala-azar sternum and spleen punctures were positive in 375 (83.3 per cent.), while in the remaining 75 cases leishmania were revealed by either one of the two methods—64 by spleen puncture and 11 by sternum puncture. In another series of 121 cases, liver puncture was added at the same time. It was found that spleen puncture was positive in 118 (97.6 per cent.), sternum puncture in 108 (89.2 per cent.), and liver puncture in 83 (78.9 per cent.).

In 45 of 88 cases under urea-stibamine therapy leishmania disappeared from bone marrow spleen and liver at about the same time after the same number of injections, as determined by means of needle biopsy while in the remaining cases the parasites always disappeared first from the liver. In another series of 203 cases, in which sternum and spleen puncture were performed to time the disappearance of the parasites, it was observed that the parasites in 88 per cent. cases disappeared from these two organs at about the same time after the same number of injections, but showed some discrepancy in time for their disappearance in the remaining 32 per cent. cases.

In following treated cases, sternum and spleen puncture were found to check up each other very closely.

## AN ACCOUNT OF BLOOD COUNT RESULTS IN SIERRA LEONE

BY

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From 1943 until the early part of 1946, 1,035 complete red cell counts were done on adult Africans at the Government Laboratory, Freetown. White cell counts were also performed on 1,005 of the blood samples.

As records of blood counts in West Africa are scanty, it is considered that an analysis of these examinations might be of interest.

The primary aim in making this survey was to see how adult African blood standards compared with European "normals," and to see what types of anaemia were prevalent in Freetown.

All the blood samples were obtained from adult African men and women attending the Connaught Hospital, Freetown, and who were referred to the Laboratory for blood counts either because anaemia was suspected or because they complained of feeling "run down."

Unlike hospital patients in England, these patients came from all social grades, from senior officials down to paupers. From the records available, it has not been possible to classify them according to their social scale. This is probably an important factor, as the diet of the educated African is of a much higher standard than that of the labourer.

The records show what standards can be expected in Africans, and also what type of anaemia was present in and around Freetown during the period under review. During the war years wages of labourers were high and the standard of living was possibly above the normal level.

It has not been possible to follow up individual cases of anaemia in detail. Experience has shown that many of the anaemias do not respond to either iron or liver preparations alone, but require a combination of both. These anaemias may be of the dimorphic type described by TROWELL (1943) in Uganda.

*Methods*—All the examinations were done by one of us or by the Laboratory Superintendent. Oxalated blood, by Wintrobe's method (1933), was used. Red and white cell counts were done in the usual way. Haemoglobin was estimated by the acid haematin method, using a Hellige haemoglobinometer which had a permanent glass standard, and was calibrated in grammes per 100 c.c. (The disadvantages of the acid method are

\* Our thanks are due to Dr W P H LIGHTBODY, Director of Medical Services, Sierra Leone, for permission to publish this paper, and to Mr S J HAWTIN, formerly Laboratory Superintendent, for help with the technical work.

recognized, but few comparisons done with Sahli haemoglobinometer and other instruments in use in the Army laboratories agreed very closely so that the degree of error if any, is probably slight.)

The mean corpuscular volume (M.C.V.) was calculated from the cell volume obtained by centrifuging the haematocrit for 20 minutes in an electric centrifuge.

Sealed wet films were also examined up to 24 hours later for evidence of sickling of the red cells.

*Normal Standards.*—The normal European standards adopted for our analysis are those given by WINTER and BURROCK (1943). They are as follows:—

Red cells 4.2 to 6.4 million per c.mm. Male average 5.5 million, female average 4.6 million cells

Haemoglobin 14 to 17 grammes per 100 c.c. Male average 15.6 grammes, female average 13.7 grammes.

Mean corpuscular haemoglobin (M.C.H.) 27 to 32  $\gamma\gamma$ . A range 29.5  $\gamma\gamma$

Mean corpuscular volume (M.C.V.) 78 to 94  $\mu$ . Average 86 c. $\mu$

White cells 4 to 11 thousand cells per c.mm.

Differential counts: Neutrophil leucocytes 33 to 75 per cent; eosinophils 0 to 6 per cent. Lymphocytes 15 to 60 per cent. monocytes 0 to 9 per cent.

### RESULTS.

Complete examination of red cells and haemoglobin was carried out on 661 adult African men and 377 women. Tables I, II and III summarize the results.

White cell counts were done on 648 men and 359 women. Table IV summarizes them.

Of the 661 men, 26 per cent. had red cell counts of at least 5 000 000 cells per c.mm. and 72 per cent. had red cell counts of at least 4 000 000. Approximately two-thirds of the latter had normal values for mean corpuscular haemoglobin (M.C.H.) and mean corpuscular volume (M.C.V.). The other third were deficient in haemoglobin in varying degrees.

TABLE I.  
RED CELL COUNTS AND HAEMOGLOBIN, MALES.

R.b.c. in millions.	Number of counts.	Per cent counts	Hb in grammes	Number of counts.	Per cent counts.	Per cent sickling.
5 and over	172	26	18+	40	31.4	29
			14-16.9	33	70.3	
			13-13.9	23	16.2	
			12—	20	11.6	
4-4.9	206	46	18+	40	6.3	
			14-16.9	43	14.7	
			13-13.9	74	34.3	
			12—	167	54.6	
3-3.9	100	17	13+	7	6.4	16
			9-12.9	60	63.3	
			9—	33	30.3	
Under 3	4	11	7-10.9	24	37.6	44
			7—	46	62.3	

TABLE II  
RED CELL COUNTS AND HAEMOGLOBIN FEMALES

R.b.c in millions	Number of counts	Per cent counts	Hb in grammes	Number of counts	Per cent counts	Per cent sickling
4.5-5.9	139	36.9	15+	15	10.8	24
			14-14.9	33	23.7	
			13-13.9	36	25.9	
			11-12.9	43	31.0	
			11—	12	8.6	
4-4.4	75	19.9	14-14.9	3	4.0	
			13-13.9	6	8.0	
			11-12.9	44	58.7	
			11—	22	29.3	
3-3.9	99	26.2	13+	1	1.0	31
			9-12.9	64	64.7	
			9—	34	34.3	
Under 3	64	17	11-12.9	2	3.1	44
			7-10.9	28	43.8	
			7—	34	53.1	

TABLE III  
SUMMARY OF MCH & MCV MALE TOTAL 661

R.b.c in millions	MCH						MCV					
	27-32 $\gamma\gamma$		27 $\gamma\gamma$ >		32 $\gamma\gamma$ <		78-94 $c\mu$		78 $c\mu$ <		94 $c\mu$ <	
	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent
5 and over	106	61.6	66	38.4	—	—	110	64.0	62	36.0	—	—
4-4.9	106	64.1	96	31.3	14	4.6	194	63.4	100	32.7	12	3.9
3-3.9	62	56.9	32	29.3	15	13.8	62	56.9	39	34.8	8	7.3
Under 3	36	48.7	25	33.8	13	17.5	29	39.2	30	40.6	15	20.2
FEMALE TOTAL 377												
4.5-5.9	89	64.0	47	33.8	3	2.2	80	57.5	59	42.5	—	—
4-4.4	45	60.0	28	37.4	2	2.6	42	56.0	33	44	—	—
3-3.9	54	54.6	33	33.3	12	12.1	47	47.5	41	41.4	11	11.1
Under 3	32	50.0	24	37.5	8	12.5	28	43.8	26	40.6	10	15.0

TABLE IV  
WHITE CELL COUNTS, MALE AND FEMALE, TOTAL 1,003

W.b.c. in thous- ands.	Per cent.		Polymorpha.			Eosinophils.		Lymphocytes.			Monocytes.	
			Per cent. normal.	Per cent. low.	Per cent. high.	Per cent. normal.	Per cent. high.	Per cent. normal.	Per cent. low.	Per cent. high.	Per cent. normal.	Per cent. high.
Under 4	41	5.8	78	17	5	78	22	90.0	8.0	8.0	85	15
4-11	803	79.0	85	4	5	65	35	91.3	1.5	4.2	82	17
11-17.9	129	1.7	74	5	22	80	20	83	18.0	3.0	76	4
Over 18	33	3.3	42	—	69	90	10	88.0	42.0	—	73	27

Of the 109 moderate anaemias, 57 per cent. were orthochromic normocytic, and of the seventy four severe anaemias 40 per cent. were orthochromic and 39 per cent. were normocytic.

Hypochromic macrocytic anaemia was about twice as common as macrocytic hyperchromic anaemia.

Of the 377 women, 58 per cent. had red cell counts of at least 4,000,000 cells per c.mm., and of these over half had normal M.C.H. and M.C.V. values. Orthochromic normocytic anaemia was found in about half of the ninety-nine moderate anaemias, and the sixty four severe anaemias. Microcytic hypochromic anaemia was about three times as common as macrocytic hyperchromic anaemia.

The sickle-cell trait was found in 27 per cent. of all the bloods examined.

Of the 1,005 white cell counts, 79 per cent. were within normal limits and in 5 per cent. a leucopenia was present. 40 per cent. of the men and 17 per cent. of the women showed an eosinophilia, and 34 per cent. of men and 42 per cent. of women had an increase in monocytes.

Among cases examined during this period, and not included in this analysis, were seven cases of myeloid leukaemia and one of lymphatic leukaemia. They were all male patients.

#### CONCLUSIONS

In many adult African men and women in the Freetown area, blood cell counts and haemoglobin levels appear to be within the normal levels accepted for Europeans.

Orthochromic normocytic anaemia is the commonest type of anaemia. Microcytic hypochromic anaemia is more prevalent than macrocytic hyperchromic.

Approximately one in four adults have the sickle-cell trait.

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## THE PROTEIN CONTENT OF CEREBROSPINAL FLUID IN TRYPANOSOMIASIS

by

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Estimation of total protein in the cerebrospinal fluid in trypanosomiasis is considered by many authorities to be one of the best guides to prognosis, a measure of progress during treatment, a method of indicating relapse or reinfection and also of cure (SICARD, 1930; ZIEHLER, 1933; FAIRBAIRN, 1934; SAUNDERS, 1944).

Of the many methods used in estimating the cerebrospinal fluid total protein, that of SICARD and CANTALOUBL (1916) enjoys considerable popularity. In this method the proteins are precipitated in a standard tube and after standing for 5 hours the precipitate is read from the calibrations on the tube. The first division on the tube indicates a total protein of 22 mg per 100 c.c., and according to SICARD and CANTALOUBL, such a protein content is to be considered as normal. The second division indicates a protein content of 40 mg per 100 c.c., and these authors consider that any cerebrospinal fluid giving a precipitate whose height is more than one and a half divisions (*i.e.*, above 30 mg per 100 c.c.) is grossly abnormal, and any between one and one and a half divisions (*i.e.*, 22 to 30 mg per 100 c.c.) is suspiciously abnormal. This is accepted by the French Trypanosomiasis Service.

On the other hand, FAIRBAIRN (1934), using this method (and also precipitation by a watery solution of picric acid) came to the conclusion that if the protein content was less than 30 mg per 100 c.c., the prognosis was good, between 30 to 35 mg per 100 c.c. the prognosis was doubtful, and above 35 mg per 100 c.c. it was very bad.

A consideration of what is considered the normal protein content of cerebrospinal fluid by other authorities (who are not necessarily concerned with trypanosomiasis) is therefore of some interest.

These have been taken at random from easily accessible sources and the methods of determination stated. Where the authors have not specified the method by which their normal figures were obtained, it has been presumed that they were by the methods described in their publications.

Authors.	Method.	Normal C.S.F. protein in mg per 100 c.
MARTINEA (1912)	Turbidometric (Disphaneometric)	12-20
BURKEL & GREENFIELD (1921)	(Mastrenat)	30
GRIFF (1930)	(Proteinometer)	40
HAERSON (1936)	(Mastrenat)	10-30
KING (1945)	(Sulphomethyl)	20-40
STITT <i>et al</i> (1945)	(Dens & Ayr)	15-40
PANTON & MURRAY (1945)	(Sulphomethyl)	20-35
HUTCHINSON & HUNTER (1946)	Precipitation (Aufrecht)	20-35
LEVISON (1929)	(Nissl's)	13-47
PURVIS STEWART (1946)	(Aufrecht)	20-30
SAUNDERS (1918)	(Scheid & Cantaloube)	22
French Trypanosomiasis Service	( )	22
Author	Turbidometric (proteinometer)	20-30 (25)

These estimates of the normal protein reveal two striking features —

1. The variability of the limits of normality given by the different authors.
2. When the same methods are used, the extreme variability in the upper limits of normal.

Both of these features depend on many factors, the main being the accuracy or degree of error of the method, and the personal factor in reading the results.

The most accurate method is obviously by the microkjeldahl, but this is impracticable for routine or field work. The Colorimetric method of Wu (Wu and LING, 1927) is very accurate but is also fairly complicated and needs a photo-electric colorimeter or photometer for readings, using 0.1 c.c. cerebrospinal fluid.

Generally speaking for routine use the two main methods are the Turbidometric (or Opacity method) and the Precipitation method.

In the latter method the height of a column of precipitate can be accurately measured (though occasional fuzziness or tilting of the "skin" sometimes causes difficulty) whereas in the Opacity method there is always great individual

variation in the assessment of turbidity. For example, in the author's laboratory, there was more often than not, when the Greif proteinometer, with trichloroacetic acid as the turbidity-producing agent (HASTON and LAIBINIS), a difference of as much as 5 to 10 mg. per 100 cc. between different readings of the same specimen.

In the light of this, the following experiment is instructive. A series of cerebrospinal fluids were examined by three different observers using the Proteinometer and Siedel and Casanovi's method simultaneously.

		Turbidity (mg. per 100 cc.)									
Dilution		1	2	4	10	20	40	2	4	8	16
Siedel & Casanovi		1	2	4	10	20	40	2	4	8	16
Proteinometer		47	23	11	5.5	2.7	1.3	2.5	1.2	0.6	0.3
Siedel & Casanovi		28	14	7.5	3.8	1.9	1	4	2	1	0.5
Dilution		1	2	4	10	20	40	2	4	8	16
Proteinometer		7	3.5	1.7	0.8	0.4	0.2	3	1.5	0.7	0.3
Siedel & Casanovi		1	2	4	10	20	40	2	4	8	16
Proteinometer		6	3	1.5	0.7	0.3	0.1	2	1	0.5	0.2
Siedel & Casanovi		7.5	3.8	1.9	0.9	0.4	0.2	4	2	1	0.5
Dilution		1	2	4	10	20	40	2	4	8	16
Proteinometer		21	10	5	2.5	1.2	0.6	2	1	0.5	0.2
Siedel & Casanovi		28	14	7	3.5	1.7	0.8	4	2	1	0.5

Major A. V. Vail, the more experienced observer in the use of the Optical method, and his results by this method showed the greatest approximation to those using the Siedel and Casanovi's tube.

In all cases, however, the Optical method appears to give protein content of cerebrospinal fluid one and a half to two times greater than by the Precipitation method.

This latter factor (which we found constantly to occur) is very important in the cases of trypanoemia, where the protein content by the Optical method is found to be between 30 and 40 mg. per 100 cc. Such a protein content according to SIEDEL and CASANOVI is grossly abnormal and according to LAIBINIS and SALTHER, has a bad prognosis, whereas according to GRUBER and others, it is within normal limits or perhaps, at the worst, at the upper limit of normal.



From these results, from a perusal of the literature and personal experience, the author is of the opinion (1) that the Sicard and Cantaloube method is relatively the more accurate method of the two main types used in routine work, in that it gives a precipitate which can be measured by a ruler rather than an estimation of opacity (2) that it would be well if authors who refer to the protein content in the cerebrospinal fluid would specify the method of estimation used.

### SUMMARY

1. The two common methods of estimation of total protein in the cerebrospinal fluid are the Precipitation (Sicard and Cantaloube) and Turbidometric (Opacity) methods.

2. Experience in the author's laboratory shows that the results given by the Opacity method tend to be one and a half to two times those given by the Precipitation method.

3. As a column of precipitate can be measured on a linear scale in the former method, this implies the elimination of the personal factor in the estimation, and therefore commends itself.

4. A perusal of the literature dealing with the normal total protein content of cerebrospinal fluid reveals (a) a wide variation of this estimation with different authors and (b) the variability of the upper limits of normal using the same methods by different authors.

It is therefore suggested that authors should always specify the methods used in their estimations.

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## THE SURGICAL TREATMENT OF THE LARGE ELEPHANTOID SCROTUM

BY

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The large elephantoid scrotum often provides better opportunity for satisfactory cosmetic results than certain smaller ones. Because of the downward traction exerted by the weight of a large tumour, the loosely attached healthy lower abdominal skin and Camper's fascia descend like an apron over pubis and penis (Figs 1 (a), 2 and 3). This thin, supple vascular sheet of ilio-pubic skin and fascia, whose lymph channels are also unimpaired, provide an excellent anterior flap for refashioning the scrotum. All tumour tissue and scrotal skin below its well defined limits of descent are excised (Figs 1c, 4 and 5).

Outside the confines of the apron, the scrotal integument is usually hypertrophied and indurated as high up as its perineal attachments. After excision of the dependent tumour mass, the anterior apron is drawn over the perineum, to which it is underslung. Its edges are sutured to the perineal margins, so covering a roughly triangular area, whose apex is directed to the perineal body (Fig 6). A circular aperture in the anterior flap provides emergence for the penis (Fig 7). The base of the preserved penile skin is sutured to the edges of the aperture (Fig 8).

In refashioning the scrotum by this method, it is desirable to utilize as much apron as possible and—as first remarked—the more massive the tumour the greater the expanse of apron.

Attempts at artistry in attaining to perfect symmetry between apposed edges are usually disappointing, one reason being that the larger anterior flap, designed to acquire a mainly spheroidal contour, has to be accommodated

within the exacting limits of a small triangular space. In fashioning a sphere suspended from triangular attachments, filling at the suture line is unavoidable. Only time, gravity and the fostering pressure of the thighs, will efface redundancies and restore normal contour.

Secondly vertically coursing vessels from the penneal branches of the external and internal pubic arteries, supplying the perineal margins, are divided too proximally to ensure a safe anastomosis at suture level. Consequently if by dint of much finicky snipping and excision of already vascularly impaired edges results appear immediately satisfactory this hard won symmetry too often gives way a day or so later to marginal sloughs, arising through poor vascularity. Edges gape and subsequently have to undergo slow union by granulation. Results ultimately as good or even better are obtained by loose approximation of skin margins. (Fig 8.)

The blood supply of the anterior apron of abdominal skin is bilaterally derived, chiefly from branches of the superior epigastric and inferior epigastric arteries as well as from the deep circumflex arteries whose cutaneous branches ramify horizontally and inoculate medially. This renders the area an ideal flap. Its generous vascularity is preserved because the horizontal incision, made at the commencement of the operation to raise the flap has not cut across the direction of cutaneous vessels (Figs. 1 (b) and (c)).

Both cords and testicles are freed from their surrounding attachments within the scrotum. Large hydroceles may have to be dealt with before replacement within the newly fashioned scrotum. After ligation of main vessels, multiple bleeding points arising after release of the tourniquet are rapidly sealed by electric cauterisation.

On completion of the operation, *tulle gras* strips are applied to the wound edges and ample dry gauze and wool dressings, firmly bandaged, are superimposed. They are left for 5 days when obvious soaking usually necessitates their removal. From this time onwards daily hypertonic saline hip-baths quickly resolve marginal sloughs.

A scrotal plaster cast, moulded at operation and lined with *tulle gras* now replaces the former dressings. This combines even pressure with comfortable support, and is changed as often as is necessary so avoiding repeated tiresome bandaging. An apron of oiled silk is worn in front over the cast to prevent soiling by urine.

Spinal analgesia with pericaine is very satisfactory in this type of operation.

The end photograph illustrates the result of an operation in 1935 carried out by this method. The excised scrotal tumour weighed 76 lb.

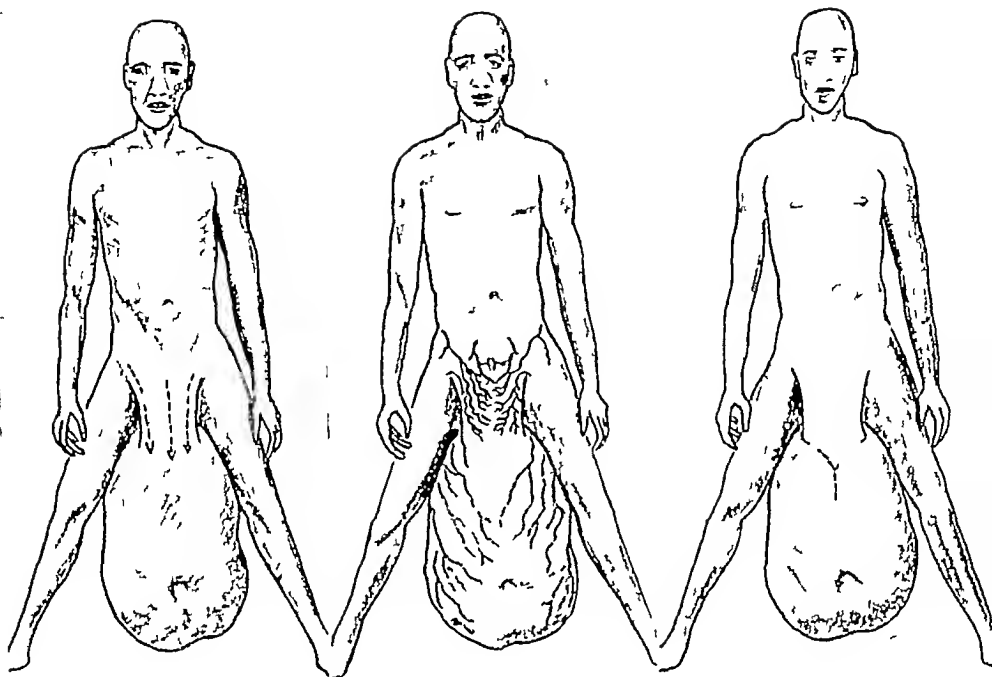
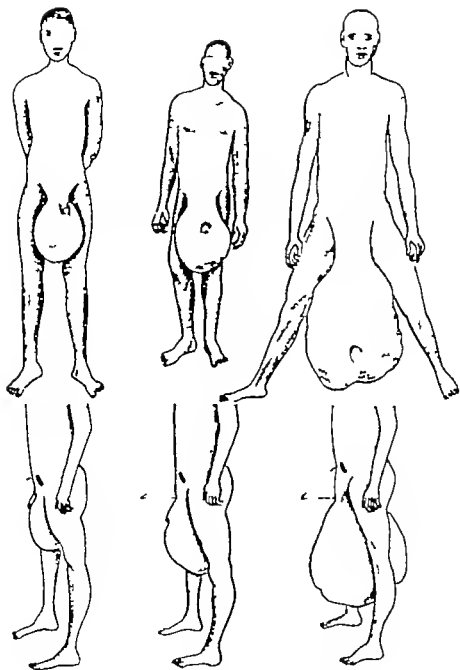


FIG 1 (a) —Showing downward traction of the lower abdominal skin over pubis and penis caused by a massive scrotal tumour weighing 76 lb

FIG 1 (b) —Showing the rich anastomosis of cutaneous vessels in the frontal apron of skin which becomes the main scrotal flap

FIG 1 (c) —Two converging incisions are made from the lower lateral borders of the frontal flap. They join below at an apex in the mid line, from which the incision is carried downwards along the axis of the buried penis. Posteriorly the incision encircles the scrotum near its perineal attachments



FIGS. 1 and 2.—Showing how the anterior apron of skin develops as result of increasing downward traction by the weight of an enlarging elephantoid scrotum.

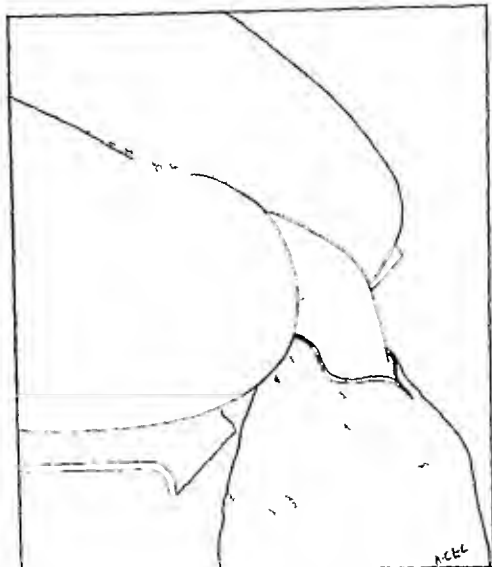


FIG 4—A superficial skin incision is mapped out with the scrotum fully dependent and before application of a tourniquet.

The incision encircles the neck of the tumour. In front it traverses the base of the healthy ilio-pubic skin apron dividing it from the indurated integument of the main tumour mass below.

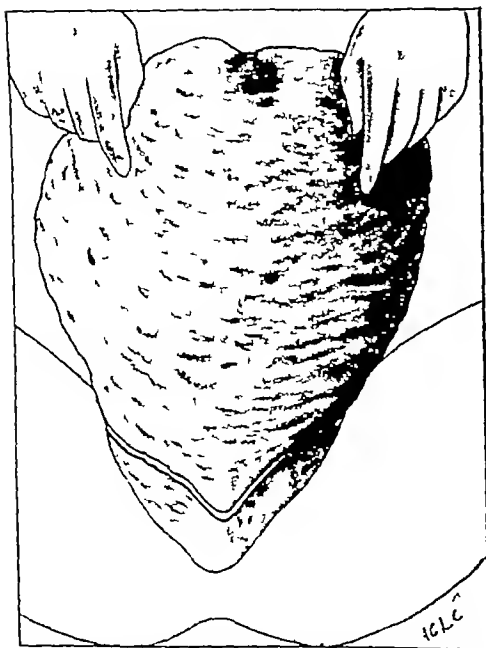


FIG 5—The tumour is raised and the superficial skin incision is continued posteriorly around the perineal surface of the scrotum.

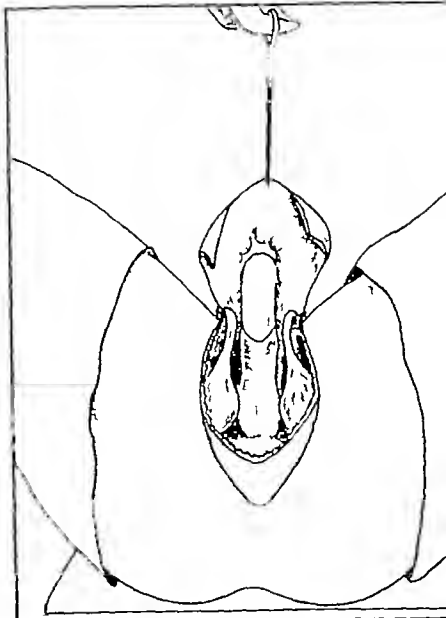


FIG 6—Upward dissection of the anterior skin flap has been completed.

An aperture has been made in it for emergence of the penis. Cords and testicles have been replaced within the open perineal scrotal pouch.

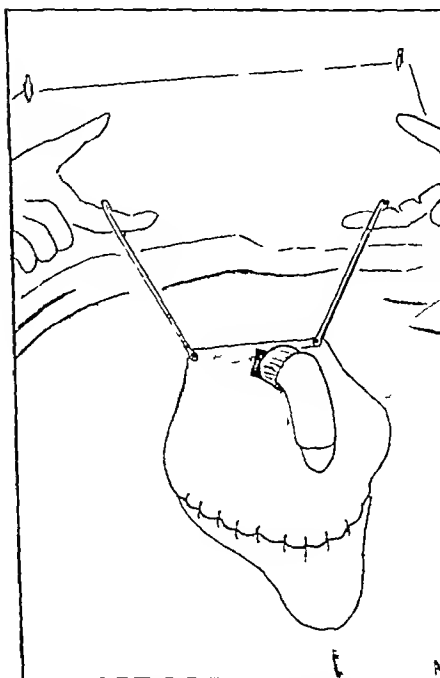


FIG 7—Showing (a) The anterior skin flap sutured to the perineal scrotal margin posteriorly. (b) Emergence of the penis through the aperture. The free edges of the restored penile skin will be sutured to the edges of the aperture.

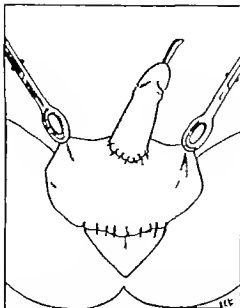
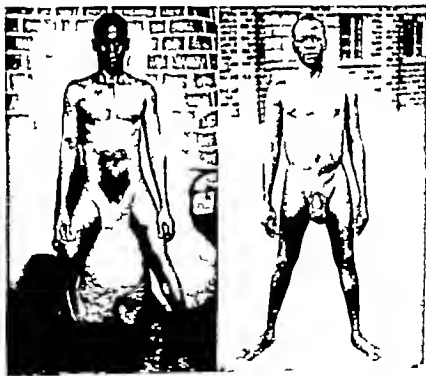


Fig. 8.—Showing the skin of the penis sutured to the edge of the aperture. Because of repeated retraction of the skin flap the aperture should not be made too high but the penis sutured in place of permanent perpendicularity corresponding with need.



Before operation weight of tumor 76 lb

After operation

## PLAGUE CONTROLLED IN HAIFA BY THE USE OF DDT ALONE

BY

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*Department of Health, Government of Palestine*

Widespread plague epidemics have been known from time immemorial. They have spread and ravished whole Continents. Populations fleeing from smitten cities have carried the disease with them. Ignorant, as they were, of its method of spread, they were powerless to combat it. Our knowledge now is such that we know where to attack the cycle of the disease at its weakest point. modern science has given us the means of attack. Although it is too early yet to draw any final conclusion from the use of DDT, sulphadiazine and streptomycin against plague, reports published so far make us hope that the world has seen its last widespread plague epidemic.

For many centuries plague has been known in Haifa. Detailed accounts are available since the British occupation in 1919. The constant finding of plague-infected rats proves that plague is endemic in this part of Palestine. The following plague-infected rats recorded in the past few years illustrates this point —

	Infected rats
1941	49
1942	31
1943	Nil
1944	157
1945	32
1946	6
1947 (All in July)	236

The actual number of positive rats is in all probability much higher than the figures would indicate because the test rat catchers employed trap over a wide area and concentrate on representative catches as opposed to large-scale extermination. In May, 1947, an unfortunate circumstance made it necessary to employ the test rat catchers on other duties and so in the outbreak about to be described the first human case of plague had occurred before we were aware that an epizootic was present in the rat population.



Haifa is an ideal location for a large rodent population to thrive. The presence of the old city so close to the port area, with large grain and food stores in the immediate vicinity makes rat extermination a formidable problem and brings the presence of plague within 100 metres of the port area, thus presenting a problem of international interest.

The old city of Haifa grew up in Turkish times and its construction is typical of the older parts of any Eastern Mediterranean town. Dwellings, shops and food stores open off small crowded courtyards where little sunshine can percolate. Each building has its dark, damp basement. Many of these at the present moment, in view of the acute housing shortage, are used for dwelling purposes. As many as four families may occupy a basement room of 8 by 6 metres. The old Turkish sewage system is the one still used. This consists of a main paved sewer running from one end of the old city to the other. It varies in height and breadth from 2 metres by 1 metre to 1 by  $\frac{1}{2}$  metre. From May till November there is never more than 30 cm. of fluid in the main channel. The sides are shelved, forming ideal rat runs and breeding places. Lateral connexions drain from the various groups of courtyards. The length of the sewer is approximately 4 km. including lateral connexions. In many instances the roof of the sewer is the flag stones paving the basement dwellings, which are broken and give easy access for rats. On account of the narrow nature of the streets and the labyrinth courtyard connexions, scavenging in the old city is a difficult task and piles of refuse form ideal feeding grounds for rats. In these ideal surroundings the rat infestation is extremely heavy. *Rattus norvegicus* is the predominant type in the whole of Haifa lower town. It is interesting to note that *R. norvegicus* is difficult to find outside the town boundaries where *R. rattus* is in the majority.

The port of Haifa is of modern construction having been completed in 1932. The port area itself is surrounded by the usual transit sheds and behind these are large commercial stores including grain and food stores. The old city comes to within 50 metres of these food stores. The modern part of Haifa has grown up all round the old city area. Immediately behind is thickly populated Hadar Hacatmel, and to the west the old city merges into the residential German colony. To the east is the industrial area with its large oil installations.

Plague starting in the old city of Haifa has the danger of immediate spread to the rest of the town. Because of the bulk of incoming commercial traffic through Haifa there is the danger of spread to the rest of the country and to the countries beyond. Syria, Lebanon, Transjordan and Iraq all receive goods which have passed through the port of Haifa. Ships leave Haifa for all parts of the world, and so international spread is a real danger.

On the 26th June, 1947 a case of bubonic plague was admitted to the Government Hospital Haifa, and the man died 2 days later. As a result of this specimen rat trapping was instituted throughout the whole of Haifa. On the 1st

July the second human case of plague was admitted showing no common factor to the first case. It was decided to start full-scale plague precautions, and after consideration a campaign was planned along the following lines —

### 1 *Immediate measures*

(a) To break the rat-flea-man chain at its weakest point by attacking the flea with DDT

(b) To limit the spread to other parts of Palestine and to take all precautionary measures to keep infected rats from boarding ships in Haifa harbour

### 2 *Intermediate measures*

Intermediate measures directed mainly against the rats

### 3 *Long-term measures*

This article will deal mainly with (1) because the immediate steps taken gave results which were dramatic beyond our expectations. In previous outbreaks thousands of pounds were spent in Haifa on anti-plague measures, yet the disease ran an uninterrupted course starting in the early summer and finishing with the advent of the rains in December. The present outbreak had every appearance of being explosive in nature, more explosive than any previously reported in this part of the world. Fourteen bacteriologically proved cases of plague, bubonic in type, were isolated in the Government Hospital, Haifa, in the first 8 days of July. In only two of these cases could a common living and working place factor be found. As well as these, six clinically suspicious cases were isolated but were never bacteriologically proved positive.

It was decided in the first phase to concentrate on laying down a DDT barrier between rat and man. The adage for our campaign was, "Widen the rat-man gap and attack within that gap." The affected area was divided into five sectors. A senior sanitary inspector was put in charge of each sector. Each inspector had working under him a DDT team laying down 5 per cent residual spray using the Four Oaks knapsack sprayer. Each squad had attached to it a number of labourers to clear impedimenta in order that the residual spray could get at dark corners and along walls liable to be rat runways. All basements, ground floors, streets, alleyways and courtyards were thus treated. This procedure was got under way on the 2nd July, the day after the appearance of the second case. A sixth team was formed to act as a mobile squad to go into action in the immediate surroundings of suspected plague cases. This squad was instructed to give heavy residual spray to the house and workplace of each suspected case, and to work in an ever-increasing circle round these localities. 500 gallons of DDT, 5 per cent residual spray, were thus laid down daily.

A propaganda campaign was instituted, using newspapers and radio. The

population was instructed to take personal precautionary measures against being bitten by fleas. They were advised to use DDT both residual spray in their houses and 10 per cent. dusting powder for their persons. Sufficient supplies of these were available in the open market. On the 4th July dusting centres were set up in the affected area. 10 per cent. DDT dusting powder was applied in these centres by using power-driven compressor blowers. The centres became very popular with the lower class Arab population, fortunately enough because it was they who were the inhabitants of the affected areas. In the first 10 days, 30,000 persons were dusted in these centres —

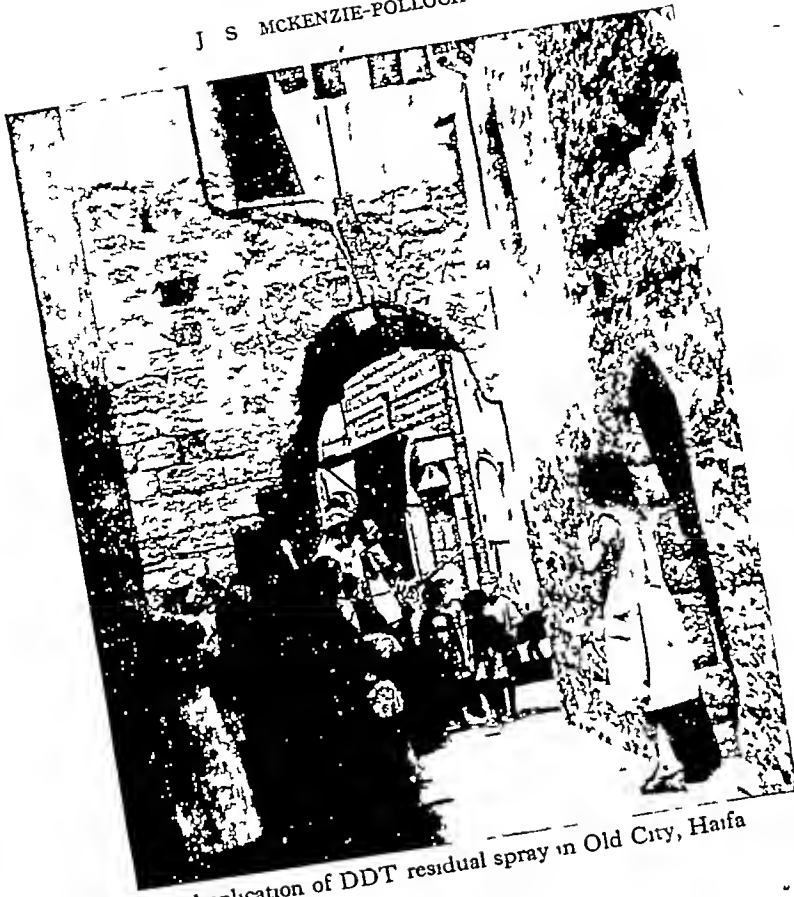
The cases in Haifa Town in 1947 appeared as follows

June 26th	1 case.	
July 1st	1 case.	
2nd	1 case.	Commencement of DDT campaign.
4th	5 cases.	
" 5th	2 cases.	
7th	4 cases.	
8th	1 case.	
" 10th	1 case.	
Total		16

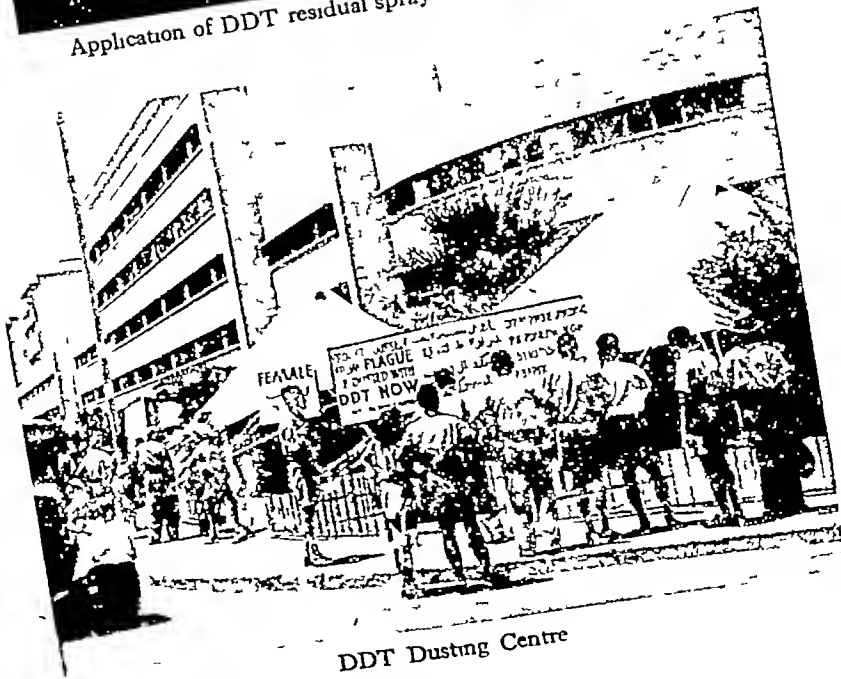
Seven days after the commencement of the extensive DDT campaign, there was a sudden cessation of human cases, although the epizootic among the rats continued with unabated severity. The following table shows the number of positive rats against the total number of rats examined in the laboratory —

Date 1947	Total examined in laboratory	Result		
		Pos.	Neg.	Decomposed.
July 1-6	19	1	1	1
13	106	61	29	1
14-20	9	35	13	21
21-7	78	31	37	10
8-Aug 3	51	9	42	
Aug 4-1	37	8	1	9
11-17	29	Nil	5	4

In the early days of the campaign a limited amount of anti plague vaccine only was available. This was conserved for persons who had been in immediate contact with plague cases, staff employed on anti-plague duties and travellers leaving Palestine. The epidemic had come under control before sufficient stocks were available to carry out wholesale immunization so it was not thought necessary to carry this out.



Application of DDT residual spray in Old City, Haifa



DDT Dusting Centre



In the first 3 days of July the *Xenopsylla cheopis* index was in the region of 3. In the 3 days from the 10th to 13th July the index had fallen to less than one flea per rat among rats caught in the affected area. (These figures are based on examination carried out by semi-skilled staff only.)

The cessation of human cases coincided so markedly with the extensive DDT programme that we must conclude that this was the agent responsible. The cessation of the epidemic was so dramatic that we have looked for some other factor that may have coincided, but without success. The epizootic continued. There was no sudden climatic change, the temperature and humidity remained normal for the time of the year and no rain fell during the whole of July.

The ease with which plague can spread outside the affected locality is illustrated by the fact that between the 15th and 18th July, four cases of plague, one of them fatal before admission to hospital, appeared in the workers of a flour mill 30 km from Haifa. Grain had been transported daily from Haifa to this mill. An extensive DDT and rat extermination programme in the mill and nearby village of Affula brought this localized outbreak to an end. In order to minimize this type of spread the following measures were carried out. All lorries transporting grain and flour were compelled to load at one of six controlled loading centres. An inspector checked each sack before loading. If the sack was damaged a thin skewer was passed through it many times to locate the possible presence of rats. The sack was then repaired before loading. This brought to light the fact that a surprising number of rats can be transported inside grain sacks. In one instance a rat's nest was found under the driver's seat in a lorry constantly used for transportation of grain. Prior to loading all lorries were dusted with 10 per cent DDT dusting powder. On being satisfied that a lorry was rat-free, a certificate was issued by the inspector. The co-operation of police road patrols ensured the checking of valid certificates on the exit roads from Haifa.

Because of the busy nature of the port of Haifa, very special measures had to be instituted to ensure that no positive rat got on board ships lying alongside. A permanent patrol ensured that all ropes connecting ship to quay were fitted with an efficient rat guard. Gangways were only allowed during hours of daylight. Loading and unloading of ships was only permitted by day. Only the captain of the ship was allowed to come ashore. Stevedores and others were dusted with DDT powder before going on board ship.

The following illustrates how important it was to insist on the strict compliance of these precautionary measures. A ship entering Haifa on inspection was found to be heavily infested with rats. An order was given that this ship should be de-ratized by cyanide gas before taking on its cargo. The ship came alongside the quay for 2 hours before being tied up at the breakwater for the de-ratization. On inspection, after the gas had been employed, 123 dead rats were found. One of these proved to be heavily infected with plague.

eliminate the psychological factor the members of this group were to be given a number of compound glucose and sodium chloride tablets, similar to that the first group were receiving. A sufficient quantity of streptomycin was available for the treatment of any possible cases from the second group. Happily circumstances made it impossible to carry out this experiment owing to the sudden cessation of cases.

In order to organize and start on a campaign of this type some days must normally elapse. Equipment, supplies and personnel are seldom immediately at hand. We were fortunate in Haifa at the commencement of this outbreak in having the complete co-operation of a well equipped and supplied Army formation. Within 24 hours of the appearance of the second case of human plague, Four Oaks sprayers in sufficient numbers were made available. Practically unlimited stocks of DDT were readily at hand. Trained Army personnel were made available to train the local workers.

It was thus possible to catch the outbreak at its absolute commencement. Such favourable circumstances must seldom present themselves.

The circumstantial evidence produced in this outbreak would indicate that by using DDT as the first line of attack, together with a modified "cordon sanitaire," rat extermination, while important, may take secondary place and should be planned on a long term continuous preventative basis.

## RESULTS OF AN INVESTIGATION OF THE THERAPEUTIC ACTION OF PALUDRINE AND PAMAQUIN ON ACUTE ATTACKS OF BENIGN TERTIAN MALARIA \*

BY

J F MONK, B M, B CH,  
*Recently Specialist Physician, R A M C*

Previous investigations have demonstrated that the addition of pamaquin to any therapeutic regime is an important factor in reducing the relapse rate in benign tertian malaria. The expected relapse rate, following treatment with paludrine alone for 10 to 14 days, is between 35 and 40 per cent. In this paper a report is given of the therapeutic effects of paludrine and pamaquin given concurrently to patients suffering from infection with *Plasmodium vivax*. The work was carried out by an Army malaria research team at the Royal Herbert Hospital, Woolwich, between October, 1945, and August, 1946.

### CLINICAL MATERIAL

The patients treated during the investigation were all soldiers returned to the United Kingdom from India, Assam, Burma and the Malay Peninsula. Approximately one-third of the cases were suffering from relapsing benign tertian malaria, the remainder presenting with a primary attack. All had had previous suppressive mepacrine therapy, and the great majority had discontinued mepacrine 4 or 5 weeks before admission to hospital. Patients who had had antimalaria treatment within 2 weeks of being admitted, were excluded from the series. Only those cases which exhibited the clinical signs of overt malaria, and in which there was no evidence of a spontaneous remission occurring, were used in the investigation.

The degree of infection of the peripheral blood was measured in all cases. The parasites were counted against 100 to 200 leucocytes in a thick, evenly

\* This work was carried out at the instigation of the Malaria Sub-Committee of the Medical Research Council. I wish to acknowledge permission from the UNDER SECRETARY OF STATE FOR WAR, the War Office (A M D 10), for permission to publish this paper, to Dr J INNES for much helpful advice, and to J C B FENTON and P B WOOD for their work on the biochemical estimations. I wish to express my gratitude. Paludrine was supplied by Imperial Chemical (Pharmaceuticals), Ltd.



spread blood film, and estimated from the total leucocyte count. The staining method employed was a modification of Field's stain with dilute Leishman counterstain as described by FENTON and LIVES (1945).

#### DOSAGE.

After preliminary toxicity trials, the following course was decided upon —  
 Course P/A Paludrine, 250 mg and pamaquin, 10 mg 8 hourly for 10 days.

This was controlled by the following standard course —  
 Course QP Quinine 10 grains and pamaquin, 10 mg 8 hourly for 10 days.

Random sampling throughout the investigation was assured by placing patients alternately on Course P/A and Course QP.

TABLE I.  
NUMBERS OF CASES TREATED.

Course.	Total number of cases treated.	Primary infections.	Relapses.
P/A	179*	63	116
QP	168	61	107

\* Includes eleven cases treated on Course P/A during toxicity trials.

Paludrine and pamaquin were given orally in tablet form. Quinine was given in a solution containing 10 grains of the sulphate to the fluid ounce.

#### THERAPEUTIC EFFECT

Rapid clinical cure was produced in all cases on both therapeutic regimes when judged by the rate of disappearance of parasites from the peripheral blood and the reduction of the pyrexia.

The average duration of asexual parasitaemia in fifty-three cases treated with Course P/A was 1.85 days, and in sixty-five cases on Course QP was 1.5 days.

The difference in the duration of temperature on both courses was found to be more significant, the average in 179 cases treated with Course P/A being 2.1 days whereas in 168 cases on Course QP the figure was 1.44 days. This bears out the clinical observation that the occurrence of a rigor expected 24 hours after the commencement of therapy was usually prevented by Course QP whereas Course P/A rarely did more than modify its severity.

J F MONK

TABLE II  
DURATION OF PYREXIA AND PARASITAEMIA

Course	Cases treated	Duration of pyrexia	Cases treated	Duration of asexual parasitaemia
		Days		Days
P/X	179	2.1	53	1.85
QP	168	1.44	65	1.5

## TOXIC REACTIONS

Toxic reactions were encountered in 7 per cent of cases treated with Course QP. Out of 163 observed cases, eleven showed a clinically detectable cyanosis with mild gastro-intestinal upset. One of these patients developed pyrexia, nausea and pain in the right hypochondrium on the 6th day, and it was considered advisable to discontinue therapy. This patient developed malaria whilst convalescent from a severe attack of lobar pneumonia. Toxic side-effects referable to quinine were negligible.

Toxic manifestations were encountered in sixty-three out of 170 observed cases (37 per cent) treated with Course P/X. Symptoms of a mild, acute gastritis were produced in a high proportion of cases for the first few days of treatment when this regime was administered to a fasting patient. Postponement of the early morning dose until after the breakfast meal greatly reduced the incidence of this side-effect which was also noticed to be related to the amount of vomiting occasioned by the malaria attack itself.

More serious toxic features were the cyanosis and anorexia which appeared on the average about the 4th day and outlasted treatment by 3 to 4 days. Thirty cases (18 per cent) displayed the cyanosis, which although rarely very marked, was easily discernible during the day-to-day observation of the patients. The anorexia and accompanying malaise were pronounced and were in marked contrast to the healthy appetite and general feeling of well-being exhibited by other patients under treatment with Course QP. It was, however, found

TABLE III  
INCIDENCE OF TOXIC REACTIONS

Course	Incidence of cyanosis	Incidence of gastric symptoms
	Per cent	Per cent
P/X	18	37
QP	7	7

unnecessary except in a single case to discontinue therapy and no ill-effects have been reported as a result of persisting in treatment.

During the investigations plasma levels of pamaquin and paludrine were estimated in a series of cases. The average plasma paludrine levels were found to be no higher than comparable figures obtained during a course of paludrine given alone in 500 mg doses 12 hourly for 14 days (ADAMS *et al.*, 1945). The plasma pamaquin levels, on the other hand were shown to be significantly higher during Course P/A than those obtained during Course QP. Independent experiments demonstrated that paludrine has an action similar to mepacrine in that it displaces pamaquin from the tissues. Since no significant toxic reactions were reported on the paludrine regime mentioned above (ADAMS *et al.*, 1945), it seems probable that the above toxic manifestations can be ascribed to pamaquin alone.

Urinary examinations were made on all cases showing toxic reactions, but in no case was evidence of haemoglobinuria or methaemoglobinuria detected. It should, however be noted that none of the patients treated belonged to the dark-skinned races in whom intra vascular haemolysis appears to be more prevalent during the administration of such drugs as pamaquin.

#### FOLLOW UP RESULTS.

The results of treatment are shown in Table IV. Patients were circled and 6 months after discharge from hospital and replies received from over 90 per cent of cases treated. The table includes for comparison the results in a series of cases treated at Woolwich with pamaquin alone (Course P pamaquin 10 mg 8 hourly for 10 days) and with paludrine alone (Course H/A paludrine 250 mg 12 hourly for 10 days). The relapse rate is expressed both as a percentage of

TABLE IV  
RESULTS OF THE TREATMENT

Course	Cases treated	Cases followed up.	Relapses.			Relapse rate.	
			Proved	Clinical	Total	Percentage of all cases treated.	Percentage of cases followed up.
P/A	179	92.7	28	9	37	20.6	22.4
QP	16	94	39	0	39	20.9	23.2
P	78	66.2	7	1	8	27.6	32
H/A	24	61.7	19	1	20	66.6	72.7

the total number of cases treated and as a percentage of those cases which were successfully followed up. The true relapse rate will lie between these figures, probably on the lower side of the mean.

### CONCLUSIONS

- 1 Pamaquin when given alone was less successful in producing radical cure over a 6-month period, than concurrent therapy with quinine and pamaquin or paludrine and pamaquin, but in a small but similar series of cases, produced a significantly lower relapse rate than paludrine given alone.
- 2 Paludrine when given alone is much less successful in producing radical cure than when it is given concurrently with pamaquin.
- 3 Pamaquin, in association with paludrine, evidently exerts a specific action in lowering the relapse rate of benign tertian malaria similar to that found when it is given in association with quinine or meperidine.
- 4 The relapse rate following treatment with paludrine and pamaquin given concurrently is similar to that following treatment with quinine and pamaquin.

Preliminary reports in a large series of cases from the L H Q Medical Research Unit (A I T), Cairns (FAIRLEY, 1946), indicate that on a similar course but with a dose of only 100 mg of paludrine given 8 hourly, not only is the relapse rate similar to that obtained with the standard course of quinine and pamaquin, but also that the occurrence of toxic reactions is negligible with this regime. It is, therefore, evident that larger doses of paludrine do not assist in lowering the relapse rate further, but merely increase the incidence of toxic reactions. A dosage of 250 mg of paludrine 8 hourly is therefore considered to be too high to be given concurrently with 10 mg of pamaquin in a 10-day course.

### SUMMARY

- 1 In a series of 179 cases treated with paludrine 250 mg, and pamaquin 10 mg given concurrently 8 hourly for 10 days (Course P/X), the relapse rate was just over 20 per cent. The results of treatment of 168 cases with quinine 10 grains and pamaquin 10 mg given concurrently 8 hourly for 10 days (Course QP) were almost identical.
- 2 The duration of temperature was appreciably shorter during Course QP than during Course P/X.
- 3 Toxic manifestations during Course QP were minimal and occurred in 7 per cent of cases. During Course P/X, 37 per cent of cases showed toxic manifestations which included cyanosis, anorexia and gastric discomfort. The clinical condition of patients during treatment with Course P/X compared unfavourably with the general good health of those on Course QP.

4 The dosage of 250 mg. of paludrine 8 hourly is considered too high to be given concurrently with 10 mg. of pamaquin, since equally successful treatment is attained with considerably lower doses which produce negligible toxic reactions.

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**Dosage**—The following two courses were used —  
 Course SN Pentaquin, 20 mg 8 hourly for 10 days.  
 Course Q SN Pentaquin, 20 mg and quinine 10 grain 8 hourly for 10 days.

Pentaquin was available as the diphosphate salt, and the dosage has been expressed as mg. of the base. It was given orally in solution to the first twelve cases treated, and thereafter in tablet form. Quinine was administered orally in a solution containing 10 grains of the sulphate to 1 fluid oz.

The number of cases occurring in the area served by the hospital was small at that time and it was, therefore, unfortunately not possible to control the investigation satisfactorily against a standard course of quinine and pamaquin, and at the same time to treat a significant number of cases.

### THERAPEUTIC EFFECTS.

Rapid clinical cure was produced by both regimes. Without a series of control cases it is difficult to compare accurately the action of these two courses, but the impression gained was that Course Q SN reduced the pyrexia more rapidly than other comparable courses. It should, however, be stated that in the majority of cases the parasitaemia was not heavy and the clinical symptoms correspondingly mild, though very persistent. It is probable, therefore, that the figures for the duration of pyrexia in Courses SN and Q SN are disproportionately low. It is certain, however, that the production of clinical cure by these two courses is no less rapid than by any other recognized form of anti-malarial therapy with quinine, mepacrine, pamaquin or paludrine.

For purposes of comparison the results of other courses administered to given in the following tables. They have been designated as follows:—

Course QP Quinine 10 grains, and pamaquin 10 mg 8 hourly for 10 days.  
 Course P \ Paludrine 250 mg and pamaquin, 10 mg 8 hourly for 10 days.  
 Course P 8 hourly for 10 days.  
 Course H \ Paludrine 250 mg 1 hourly for 10 days.

TABLE I. DURATION OF PYREXIA

Course	SN	Q SN	QP	P \	P	H \
				21	28	21
			144	(178 cases)	(22 cases)	(24 cases)
Average in days	1.2 (25 cases)	0.72 (78 cases)				

### TOXIC REACTIONS.

Toxic reactions were frequent during both courses, nearly half the cases either becoming cyanosed or developing gastric symptoms. Cyanosis appeared usually about the 3rd or 4th day, and outlasted treatment by 3 to 4 days. It showed as a dusky grey colour, most marked in the lips and conjunctival mucous membranes and was accompanied in the majority of cases

TABLE II INCIDENCE OF TOXIC REACTIONS

Course	Cases treated	Number of cases showing toxic signs		Total number of cases showing toxic signs
		Cyanosis	Gastric disturbances	
SN	25	7 (28%)	7 (28%)	11 (44%)
Q/SN	26	12 (46%)	8 (30%)	13 (50%)
QP	163	11 (7%)	8 (5%)	18 (11%)
P/A	170	30 (18%)	43 (25%)	63 (37%)
P	29	Nil	1 (3.5%)	1 (3.5%)
H/X	24	Nil	1 (4%)	1 (4%)

by gastric disturbances. Nausea and anorexia were marked, and there was complaint from several patients of epigastric pain and flatulence. One patient on Course Q/SN developed cyanosis on the 4th day of treatment, and by the 6th day there was marked tenderness under the right costal margin, nausea, anorexia, and central abdominal pain of a sharp and continuous nature. The liver was not palpable, and the urine showed no abnormality. At the same time the temperature rose above 102° F, although the patient had been afebrile for the previous 5 days. The peripheral blood remained free from parasites. These signs and symptoms, except for the cyanosis, had disappeared within 48 hours of discontinuing treatment, and did not reappear during the subsequent administration of a 6-day course of quinine and pamaquin. The cyanosis gradually faded during administration of the latter course. The clinical evidence in this case pointed to some hepatic disturbance as well as gastro-intestinal upset.

SCHMIDT *et al* (1945) showed that the toxicity of pentaquin in monkeys was approximately one-quarter that of pamaquin. In man it appears to be more than half as toxic whether given alone or with quinine. ALVING (1946), however, treated patients with a course similar to Course Q/SN for 14 days, and has reported very few toxic reactions. LOEB (1946) concludes that "the daily dose of 60 mg should not be exceeded". It appears from this series that 60 mg per day is probably too high a dosage for routine treatment. Extensive and controlled therapeutic trials will be necessary before an optimum dosage of pentaquin can be recommended.

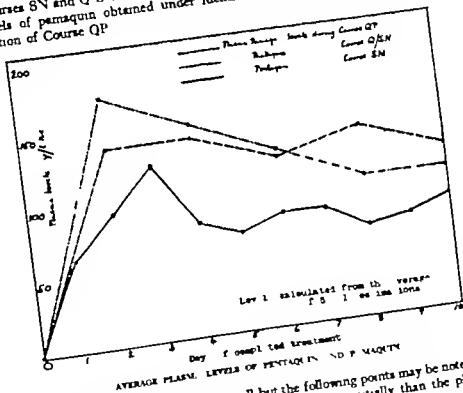
#### PLASMA LEVELS

The plasma levels of pentaquin were estimated on fourteen cases treated with Course SN and ten cases treated with Course Q/SN. The method used was a modified form of that described for pamaquin by BRODIE *et al* (1945).

When the plasma level is measured after a single dose of 20 mg pentaquin,

or after 3 or 4 doses given at 8 hourly intervals, it is found that a stable level is only reached after a minimum of 6 hours, and probably not until at least 8 hours after the last dose. At such times the level is frequently too low to be estimated accurately by the method employed. It therefore became necessary to estimate the peak plasma concentrations which occur approximately  $2\frac{1}{2}$  hours after administration of the drug. Although peak level estimations tend mainly to reflect the rate of absorption of a drug some indication is also given of the degree of saturation of the tissues and cells unless there is considerable change during a course of treatment in the rapidity of absorption from the alimentary canal or into the tissues.

The average plasma pentaquin levels found during the administration of Courses SN and Q SN are shown in the graph, in comparison with the plasma levels of pamaquin obtained under identical conditions during the administration of Course QP.



The numbers of cases utilized are small but the following points may be noted —

1. The plasma pamaquin levels are higher initially than the plasma pentaquin levels of either Course SN or Course Q/SN
2. The plasma pentaquin levels during Course Q/SN are higher on the average than during Course SN
3. A stable plasma pentaquin level is reached by approximately the

3rd or 4th day of treatment A stable plasma pamaquin level is not reached until about the 8th day of treatment

This last point is probably of considerable importance Pamaquin is readily broken down and rapidly disappears from the peripheral circulation when given at 8 hourly intervals It is probable that significant amounts are not present in the plasma 8 hours after the last dose until towards the end of a 10-day course of treatment This is indicated by the fact that on the average a stable pamaquin level is not reached in the plasma until about the 7th or 8th day of treatment, as shown by the flattening-out of the curve in the graph This conclusion is also supported by the figures obtained from individual cases

Pentaquin, on the other hand, probably reaches a stable level in the plasma as early as the 3rd day and certainly by the 4th day of treatment It therefore follows that a significant amount of the latter drug is maintained in the plasma between the 8 hourly doses for a period of 4 or 5 days longer than is the case with pamaquin

That it is so maintained is also demonstrated by the fact that although it is readily broken down and rapidly disappears from the plasma during the initial administrations, it is yet recoverable from the peripheral circulation for a considerable period after discontinuing treatment In one case detectable amounts were present 4 days after the last dose had been given, and in a large percentage of cases, after 48 hours The plasma rarely shows significant amounts of pamaquin to be present longer than 36 hours after discontinuing treatment with quinine and pamaquin

If, therefore, it is assumed that a stable level of the drug in the plasma indicates a stable level in the tissues, and that this latter fact is of importance in obtaining a radical cure of benign tertian malaria, then pentaquin would appear to have an obvious advantage over pamaquin when the drugs are administered at 8 hourly intervals

#### FOLLOW-UP RESULTS

The results of treatment are shown in Table III Patients were circularized 6 months after discharge from hospital, and the relapse rate obtained has been expressed both as a percentage of the total number of cases treated, and as a percentage of the number of cases successfully followed up

It will be seen from Table III that Courses SN and Q/SN give a smaller relapse rate than any of the other courses shown The figures of cases treated are small so that the degrees of success of the various regimes are not strictly comparable The results do, however, suggest that extensive trials with pentaquin may prove considerably more successful than other treatments yet tried on a large scale It should also be noted that the strains of *Plasmodium vivax* causing the attacks were from the same geographical areas for all six courses

The relapse rate estimated for Course Q/SN is probably misleadingly high The worst possible interpretation was placed on the replies received from the

TABLE III. RESULTS OF TREATMENT

Course.	Number of cases treated.	of cases followed up	Relapses.				
			Proved.	Clinical	Total	Percentages.	
						Of all cases treated.	Of cases successfully followed up.
RN	1	8%	2	1	3	1.0	13.4
Q SN	46	9%	0	3	3	11.5	12.8
QP	18	4%	20	8	28	50.0	23.2
P \	179	25%	25	9	34	20.0	22.4
P	79	25%	7	1	8	27.4	22.0
H \	4	8%	16	1	17	64.8	72.1

three patients to whom clinical relapses had been ascribed. Treatment was self-administered in two of the cases merely for symptoms of a mild fever of unknown origin. The third case was treated by his own doctor for more severe symptoms with some suggestion of a rigor but there was no history obtainable from the other two cases suggestive of rigors, and none of these patients reported an alternate-day type of fever.

## SUMMARY

In a series of twenty five cases of benign tertian malaria treated with pentaquin, 20 mg. given 8 hourly for 10 days (Course SN) the relapse rate after a 6-month period was 12 per cent of all cases treated. The equivalent figure for a course of pentaquin, 20 mg. given concurrently with quinine 10 grains, 8 hourly for 10 days (Course Q SN) was 11.5 per cent.

Rapid clinical cure was produced by both therapeutic regimes and comparison is made of the duration of pyrexia between these two and other courses.

Toxic reactions during Course SN and Course Q SN manifested themselves in nearly 50 per cent. of the cases treated. It appears that 60 mg. of pentaquin daily is too high a dose to be given routinely without further extensive trials. The significance of the plasma levels of pentaquin during treatment are discussed.

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## QUININE BY CONTINUOUS INTRAVENOUS DRIP IN THE TREATMENT OF ACUTE FALCIPARUM MALARIA

BY

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During the years of the Japanese occupation of Malaya, the writer had the opportunity of treating, observing or scrutinizing the case records of nearly two thousand cases of acute *Plasmodium falciparum* malaria in the malnourished British, Australian and Dutch prisoners of war in Changi, Singapore

Treatment was based on quinine followed, as long as stocks were sufficient, by atebrin and plasmoquine. Severe infections received quinine by intramuscular or intravenous injection. For the first 2½ years, intravenous quinine was given almost entirely by standard methods, but for the last year there was a change over to a continuous intravenous drip technique. This change, adopted on account of fatalities apparently related to the standard intravenous technique in use, was associated with the recovery of cases which under standard treatment we should have regarded as hopeless.

This paper records clinical and parasitological details of fifteen cases treated by this drip technique.

INTRAVENOUS QUININE, FEBRUARY, 1942, TO SEPTEMBER, 1944

There were approximately 1,000 admissions to hospital for acute falciparum malaria during the period February, 1942, to September, 1944. The majority of these cases occurred in 1942 in one of the worst periods of malnutrition. No record is available of the number of intravenous injections which were given, but it was small. The quinine solution for injection contained 1 grain

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of the bishydrochloride to 2 c.c. of sterile saline the rate of injection was 2 c.c. a minute and the usual single dose 0.5 to 0.66 gramme.

The following four fatalities, apparently associated with this method, are recorded —

TABLE I

Species of <i>Plasmodium</i>	Intensity of infection	Notes.
<i>falciparum</i>	Heavy	Intravenous injection, 0.5 gramme. Icteric convulsions and death.
<i>falciparum</i>	Very heavy	Intramuscular quinine, 0.66 gramme followed 1 hour later by intravenous quinine, 0.2 gramme; 20 minutes later collapse and death.
<i>falciparum</i>	Moderate	Died during an intravenous injection.
<i>falciparum</i>	Heavy	Intravenous quinine 1.0 gramme in 2 c. water. Icteric epileptiform convulsions. Death.

In addition to these deaths, here was one of severe malaria in which the patient, during course of arsenic therapy developed an epileptiform fit and died immediately after an intravenous injection of 0.66 gramme of quinine.

### INTRAVENOUS QUININE OCTOBER, 1944 TO SEPTEMBER, 1945

There were approximately 1 000 cases of acute falciparum malaria admitted to hospital in this period. It was a period of considerable malaria incidence in a small centralized camp during the worst period of malnutrition. Facilities for diagnosis, observation and treatment were better than in the early confused period of imprisonment and it was possible to carry out the routine as hereafter described.

#### (a) CRITERIA OF SEVERITY OF INFECTION REQUIRING DRIP TREATMENT

As a rule all infections with over 100 parasites per thick film field (Field's method), were given a preliminary intramuscular injection of 0.5 gramme of quinine, whatever the clinical condition might be. When the thick film field showed that there were considerably more than 100 parasites per field, a parasite count was made. Where the count was over 300 000 parasites per c.mm. (estimated by total red cell count and the number of trophozoites per 100 red cells) intravenous drip quinine was given regardless of the clinical condition. It was also given in cases where cerebral or other pernicious signs were present or developed in the course of the attack.

CLINICAL NOTES

CLINICAL DETAIL OF 15 CASES OF ACUTE FALCIPARUM MALARIA														
No.	DAY	PERIPHERAL INTENSITY OF PARASITES PER CMM					DAY	QUININE TREATMENT					REMARKS	
		1	2	3	4	5		6	7	8	9	10		
1	+++						1							YEAR COMA ON 3 P MORNING BECAME WOODS. DIED STARTED DEATH IN TONIC SPASM 3 HOURS LATER.
2	336,000	++					2							ADMITTED IN EVENING HAD HAD 2 1/2 QUININE PREVIOUS TWO DAYS. STUPOROSE. HYPOTENSIVE. PNEUMONIA. BLOOD AND BLOOD CELLS 1,000,000. RECOVERY.
3	124,000						3							DEVELOPED SEVERE CRYSTALINITY. BACILLI. MENTAL COMA AND COLLAPSE. RECOVERY.
4	343,000	+++					4							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
5	411,000	+++					5							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
6	384,000	++++					6							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
7	318,000	504,000	+++				7							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
8	971,000	800,000	275,000	+++			8							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
9	642,000	480,000	73,000	3,000	+++		9							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
10	884,000	783,000	162,000	+++	+++		10							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
11	++++	++++	++++	++++	++++		11							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
12	++++	116,000	240,000	++++	++++		12							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
13	164,000	592,000	135,000	41,000	500		13							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
14	482,000	++++	7,000	+			14							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.
15	987,000	+++	+++	+++			15							ADMITTED WITH ORDEMA. ACUTE. AND HYPOTENSIVE. PNEUMONIA. RED BLOOD CELLS 1,250,000.

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## (b) TECHNIQUE \*

*Apparatus*—Soluvac or vacolite gravity flask, with drip regulator, and tied-in cannula

*Vehicle*—Sterile normal saline, sometimes with an intravenous preparation of thiamin added

*Dosage*—2 grammes of quinine bihydrochloride in 24 hours, 0.5 to 0.66 gramme per litre

*Rate of flow*—30 to 40 drops per minute

*Site of injection*—Long saphenous vein at ankle, to allow use of arms, leg placed on short back splint with footpiece to immobilize foot

## CASE RECORDS

The parasitological and clinical details of the fifteen cases so treated are set out in Table II

The drip was continued for 12 hours to 4 days, depending on the severity of the infection, the condition of the patient and as experience gave confidence in the method. The advantages of this method were —

(a) The ease with which it could be combined with other treatment. For instance, in Cases 2 and 7, 600 c.c. of blood were given for gross anaemia. In Cases 7, 10, 11 and 14, thiamin was given for actual or suspected deficiency, and in Case 12, on the 3rd day of treatment, for acute cardiac beriberi.

(b) The certainty of complete absorption in any case. In the state of malnutrition of many cases, failure to absorb drugs was a real danger.

(c) The certainty of a constant safe concentration of quinine without risk of degradation products reducing its efficiency.

(d) The absence of unpleasant general effects.

The disadvantage was —

(a) Some mild phlebitis of the vein when the cannula was *in situ* for 2 to 3 days, with risk of severe septic phlebitis.

## THE RECOVERY OF HYPERINFECTIONS WITH CONTINUOUS INTRAVENOUS QUININE DRIP THERAPY.

FIELD (1937), in an analysis of parasite counts made on the 1st day of treatment in a series of 750 cases of acute falciparum malaria, concluded that the extreme limit of tolerance of Asiatic adults for Malayan strains of *P. falciparum*, seemed to be in the region of three-quarters of a million parasites per c.mm. of peripheral blood. Further observations by the same author in a series of 2,000 cases record no recovery where the count exceeded this figure.

\* The writer is indebted to Major R. DICK, A.A.M.C., for this description.

(FIELD, unpublished). In the series of cases now under discussion it will be noticed that Cases 8, 10 and 13 with counts of 800,000 to 1,240,000 parasites per c.mm., recovered from their attack, although in Case 13 death supervened as a result of sepsis a constant danger under the prevailing conditions.

### COMMENTS.

For the last quarter of a century the fall of blood pressure, associated with intravenous injection of quinine, the duration of the cardiac depression and the necessity to control the injections by blood pressure observations when the general condition is poor and the blood pressure low have been recognized. (McCARRISON and CORNWALL, 1919) HEILIG and VISVESWAR (1944) report that in fourteen of twenty four malaria patients who were given a total of 2.68 grammes of quinine by a daily intravenous injection in 10 c.c. of water over 5 days myocardial impairment (measured by electrocardiogram readings) appeared after the fifth injection. Depression of the R-T segment and the reduction or the abolition of the T wave is also recorded (HIVONA, 1931). The mechanism of the circulatory depression caused by quinine is discussed by DREINBACH and HANZLIK (1945), who remark that in serious cases of collapse in man, the cardiac poisoning may be irreversible and all treatments may be of no avail. They record an interesting but unexplained effect of thiamin as an antagonist to quinine depression. There is reason to believe that the lack of thiamin in the camp diet played some part in the instability of the cardiovascular system. Prisoners of war were in a state of semi-starvation, thiamin and protein deficiency. The cardiovascular system was unstable and hypotension was general. There were sudden deaths due to circulatory failure in cases of chronic malarial infection and other diseases, deaths which are seldom seen under normal conditions and which are outside the scope of this paper.

A similar experience of the dangerous effect of intravenous quinine injection is recorded from the General Hospital, Colombo, during the great epidemic of 1935 (DE SILVA, 1935). The association of starvation with malaria was a feature of this epidemic, and it was found that intravenous quinine was contra-indicated where the systolic pressure was below 90 mm. Hg (FERNANDO and SANDARASAGARA, 1935).

Many workers have used successfully intravenous quinine in high dilution in normal or glucose saline in the treatment of pernicious malaria (JAMES, 1913; WRIGHT 1915; THOMAS and STROENSTRICKER, 1938; STIMPSON and SARGENT, 1943; FITZ HUGH, PEPPER and HOPKINS, 1944). These workers used doses of from 0.32 to 2.0 grammes in 250 to 500 c.c. or more given slowly and in some cases repeated at 6 to 8 hour intervals till pernicious symptoms had disappeared and oral therapy could be started. Dr R. B. HAWES, one time Professor of Medicine, Singapore taught students to give into the ankle vein, controlled by a c.p., quinine 0.68 grammes in 1 pint of normal saline once only to cerebral cases. LINDLEY (1943) advocates quinine 0.4 grammes in 1 pint of

normal saline in algid cases. The Office of the Surgeon-General of the U S Army, Circular Letter No 153, recommends 0.66 gramme in 300 to 400 c c saline, repeated if necessary at 6 to 8 hour intervals, although for vomiting it recommends continuous drip of dextrose with thiamin added. HANZLIK and CUTTING (1945), after clinical trials with quinine-epinephrine, recommend 0.5 gramme quinine and 1 mg epinephrine in 250 c c isotonic sodium chloride injected in not less than 30 minutes, with not more than two or three injections properly spaced within 24 hours.

The fall of blood pressure is stated to be less with dilute infusions (BRAMACHARI, 1922), although this observation is not supported by the work of MCCARRISON and CORNWALL (1919). The writer has tried to trace in the literature reference to the use of the continuous intravenous drip method of injecting quinine, and can find from the resources at his disposal (sadly depleted as the result of war), only three references.

BRATTON (1945) has reported a method of continuous intravenous chemotherapy of *Plasmodium lophurae* infection in ducks to avoid the difficulties of incomplete absorption, and of possible degradation of drugs in the alimentary canal when administered by mouth. A similar method was used in an Indian hospital during the war, but it is stated that the hospital was too busy to appraise its value (KAHN, 1945). DON and MEYER (1944) report a case of cerebral malaria with 35 per cent infected cells, which after 3 days' treatment passed into coma, and after 24 hours on a continuous drip containing 2.0 grammes of quinine recovered in spite of an attack of blackwater fever.

The series of cases described appears to afford evidence that this method of using intravenous quinine in a dilute form sufficient to secure administration of the normal 2.0 grammes of quinine in 24 hours is a safe and effective method.

#### SUMMARY

- 1 This paper records the treatment by a continuous intravenous quinine drip technique of fifteen cases of heavy *P. falciparum* infection in malnourished prisoners of war in a Singapore camp. These cases were selected from a series of approximately 1,000.
- 2 The efficiency of the method, its simplicity, and the ease with which it can be combined with blood transfusion or the slow administration of thiamin are stressed.
- 3 Recovery by this method of treatment is recorded of three cases with a peripheral intensity of infection higher than has hitherto been reported in Malaya with survival.
- 4 The author is of the opinion that this is a safe and effective method for the treatment of pernicious *falciparum* infections.

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# FAILURE OF NEO-ARSPHENAMINE IN RELAPSING VIVAX MALARIA.

BY

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R STEWART JOHNSTON

AND

HUGH SMITH

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The civilian internment camp in Changi Gaol, Singapore, was moved in May, 1944, to huts in open country a few miles away. Within a fortnight cases of *Plasmodium vivax* malaria appeared in a population of about three thousand hitherto almost malaria-free. This early intimation of trouble ahead heralded a sharp vivax epidemic with over five hundred cases in a few months. There was enough quinine, but little else. Relapses were frequent, and we heard almost daily the melancholy story of attack after attack, controlled only by the suppressive therapy that we were able to give to none but the fortunate few.

A small package of neo-arsphenamine had been found by one of us in an Australian dump\*. There was enough to give two injections to each of twenty relapsers on the following scheme —

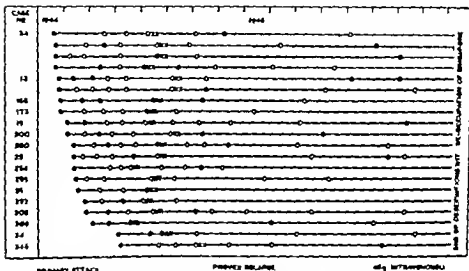
A small package of quinine was given to the Australian dump * There was enough quinine for 10 cases With more hope than confidence, we gave the relapsers on the following scheme —										
Quinine 10 gramme	Quinine 10 gramme	NAB 0.45 gramme	-	-	-	-	-	-	-	NAB 0.45 gramme
0	1	2	3	4	5	6	7	8	9	10
Day after relapse										
These cases was good They lived with September, 1945 TH										

The opportunity for watching these cases was good. They lived with us and we observed them until the camp broke up in September, 1945. There

\* Neo-arsphenamine (May & Baker)

were good facilities for microscopic diagnosis. The chances of fresh infection at the time when the injections were given and afterwards were small. Based on the population at risk and the incidence of new infections in this small and nearly closed community the chance of re-infection in any one month ranged from 1 in 85 to 1 in 1,500.

The later vivax history of these twenty cases is shown in the accompanying figure. Nineteen of the twenty infections held their own with scarcely a ripple in the relapse rhythm.



Twenty cases of relapsing vivax malaria treated with two injections of neo-arsphenamine

**Comment.**—Relapsing *P. vivax* malaria is one of the minor but troublesome legacies of war. Many thousands of cases will pass through the hospitals of Great Britain and America over the next few years. They will be given drugs and drug combinations in great variety. We trust that the fortune of those who pin their faith on neo-arsphenamine will be better than ours.

# PHLEBOTOMUS IN NEW GUINEA AND NEARBY ISLANDS

BY

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AND

OWEN H. GRAHAM, Major, SN C, A U S

During World War II, United States and Australian troops in the Southwest Pacific developed fevers of 2 to 4 days' duration and, since a great many were not diagnosed, they were listed as fevers of undetermined origin. Although it was believed that some of the men may have had atypical dengue or partially suppressed malaria, the suspicion arose that some might be suffering from sand-fly fever. Consequently, surveys were made to determine the presence and distribution of phlebotomus at bases in New Guinea and neighbouring islands. This report deals with such studies made by the authors during 1944.

Species of *Phlebotomus* have been described from Australia and the Dutch East Indies, but none has been described from New Guinea (TONNOIR, 1935). However, in 1917, this midge was shown to occur there when specimens were collected at Port Moresby, Papua, by Dr W M STRONG (1921). These specimens, two females, were forwarded to the United States by Dr S M LAMBERT, International Health Board, Rockefeller Foundation, who was in Papua carrying on hookworm studies. In a personal communication to the writers (31st August, 1944), Dr LAMBERT stated that the phlebotomus specimens were turned over to the late Dr FRANCIS M ROOR, of Johns Hopkins University, for study, and quotes the following sentences from Dr ROOR's report: "The vial contains two specimens of a species of *Phlebotomus*. So far as I can learn, no species of this genus have been described from the Australian region. These specimens probably belong to an undescribed species though, since the



character of last resort in the *Phlebotomus* is the male genitalia, no exact determination can be made from females alone. The final disposition of these specimens has not been determined. HILL (1923) states that during a recent visit to Papua (presumably during 1922), Dr SIMON placed his collection at my disposal and also searched for fresh specimens, but further examples were not secured. He goes on to say that shortly after his return to Australia, Dr SIMON collected and sent him one female from Papua. This specimen was not described but was found to be closely allied to females of the first species to be described from Australia, *P. guerrislandi* Hill, 1923.

As far as can be determined, no further collections of phlebotomus have been reported from this part of the Pacific with the exception of those made



FIG. 1. Illustrating the use of the frozen aerosol bomb (insecticide dispenser) in collecting phlebotomus.

in Australia (TOWNSEND, 1933). Australian and American Army medical men were, therefore, greatly interested when it was shown in 1944 that phlebotomus is apparently very prevalent in New Guinea and neighbouring islands.

#### COLLECTION METHODS.

HERRI (1945) states that during the day adults of phlebotomus seek variety of resting places: in dark corners and near the ceilings of buildings and, on the outside, in masonry cracks in stone walls, excavations, animal burrows, hollow trees, deep cracks in the soil and on tree buttresses. Earlier HERRI (1942) reported that tobacco smoke is of great value in searching daytime shelters. Smoke causes the sandflies to move and thus reveal themselves, and temporarily dulls their reactions, making them easy to catch.

In letter to the writers in early 1944 HERRI suggested that the jungle in New Guinea might prove to be fertile habitat in which to collect phlebotomus. During July 1944 the first specimens were collected. Dobodura, Papua, by blowing tobacco smoke into the tree hole shown in Fig. 1.

Using smoke, further collections were made from tree buttresses and trunks as well as holes. Later it was found that the freon aerosol bomb (insecticide dispenser) being issued by the Army for control of adult mosquitoes in tents and buildings, was of great value in collecting sandflies rapidly. As illustrated in Fig 1, a white cloth was spread at the bottom of a tree hole or on the ground between buttresses and around the tree trunks. The holes or bark were liberally sprayed with the insecticide. A host of flying insects would usually become active and then, due to the effects of the insecticide, soon begin to drop on the white cloth and die. Using this method, sometimes several dozen sandflies were collected from a single tree hole or buttress.

Although all our collections were made in the jungle from tree habitats, it appeared likely that, because of the brief activity of the sandflies before death, the freon aerosol bomb could be used to advantage in collecting phlebotomus from masonry crevices, cracks in the soil, etc. However, it soon became apparent that this method of collection had one serious disadvantage. Once the insecticide had been sprayed on a buttress or tree hole, phlebotomus was seldom, if ever, again collected at that site. Apparently there was a residual effect that repelled or caused the death of any sandflies that remained or came to these sites from elsewhere. If live sandflies are to be collected, the bomb cannot be employed, and then tobacco smoke should be used since its effects are only temporary.

### COLLECTIONS

All our specimens of phlebotomus were collected from tree holes, trunks, or buttresses. None was ever taken in foxholes or pillboxes. Fallen hollow trees never yielded specimens. However, numerous representatives of other species of the family Psychodidae were noted in these locations. Light traps set up near tree buttresses from which phlebotomus could be collected never yielded sandflies but other members of the family were sometimes numerous. HERTIG (personal communication) states that in Panama a few specimens have been taken in light traps.

The table lists the various Army bases, localities within these bases, and islands, where we were able to collect phlebotomus. The locations of these bases and islands are shown on the map (Fig 2).

Representatives of all these collections were forwarded in September, 1944, to Mr D J LEE, Department of Zoology, University of Sydney. Recently, the remainder of our specimens have been turned over to Major MARSHALL HERTIG, SN C, A U S, Gorgas Memorial Laboratory, Panama, Republic of Panama. In a preliminary report on his studies, Mr LEE states, "An examination of this material has shown that all forms so far seen are undescribed species, and so far all belong to the recumbent haired group." He indicates that two or more species are represented. It is anticipated that LEE or HERTIG will eventually publish descriptions of these sandflies.

### DISCUSSION

Due to the increasing tempo of the war in late 1944, and the invasion of the Philippines, it was not possible to continue working with phlebotomus. Nothing was determined regarding the feeding habits of the sandflies at any of the various bases where collections were made. HERTIG (personal communication) states that in a dense Panamanian forest two men collected over a

TABLE  
DATA CONCERNING COLLECTIONS OF *Phlebotomus*.

Place collected.		Habitat of sandflies.	Number of collections.
Army base.	Locality		
Oro Bay	Dobodura	Tree holes and buttresses	11
	Popondetta	Tree buttresses	4
Port Moresby	13-mile swamp		1
Lae	Lae		1
Nadzab	Nadzab		2
	Nerakapori village	Tree holes and buttresses	2
Finschhafen	Mape River	Tree buttresses	1
	Base F Headquarters		1
Saidor	Wab Beach		1
Aitape	Koraka		1
	Tumleo Island		1
Hollandia	Hollakong		1
	Bewani Jerry		1
Tomia	Arare		1
Owi Island	Owi Island		1
Baik Island	Molmer Drums	Tree holes and buttresses	1
Noemfoe Island	hornsorens	Tree buttresses	1
Seusepor	Mar Village		1

hundred sandflies in the act of biting them. It is reasonable to expect that some forms from the New Guinea jungle will do likewise.

What role, if any *phlebotomus* may play in disease transmission in New Guinea remains for future studies to reveal. In view of the experience of troops with fevers of short duration and the abundance of sandflies the problem must remain an open one. Sandfly fever has not been reported from Australia. However HILL (1923), in his paper describing the first species from Australia, *P. Queenslandi* writes, "The occurrence of some undetermined fevers in North Australia naturally suggests the possibility of dipterous intermediaries occurring amongst the numerous species of Chironomidae, Psychodidae and Simuliidae, but so far no evidence has been obtained to incriminate any of these flies. The species described in this paper has well-developed biting mouthparts in both sexes and is, therefore, a potential disease carrier." It was reported by TONKOWA (1935) that the Australian species *P. brevifilis* does feed on human blood. As far as can be determined, it has not been incriminated in disease transmission.

Concerning the possible presence of sandfly fever in the Pacific, it was of interest to read FARNER's paper (1944), in which he refers to "Die deutschen Marianen ihre Natur und Geschichte," a publication of Von PROWAZEK (1913).

Von PROWAZEK mentions "Saipan influenza" or "missilepik" It was described as similar, and possibly identical, with sandfly fever He predicted that *Phlebotomus* would ultimately be discovered in the Marianas FARNER states that extensive entomological collections in these islands have failed to detect the presence of any species of this genus, and it appears extremely unlikely that missilepik is phlebotomus fever It was possible for one of the writers to visit Guam in March, 1945 A limited search was made for phlebotomus, and no specimens were found However, there had been little rain for some time and,

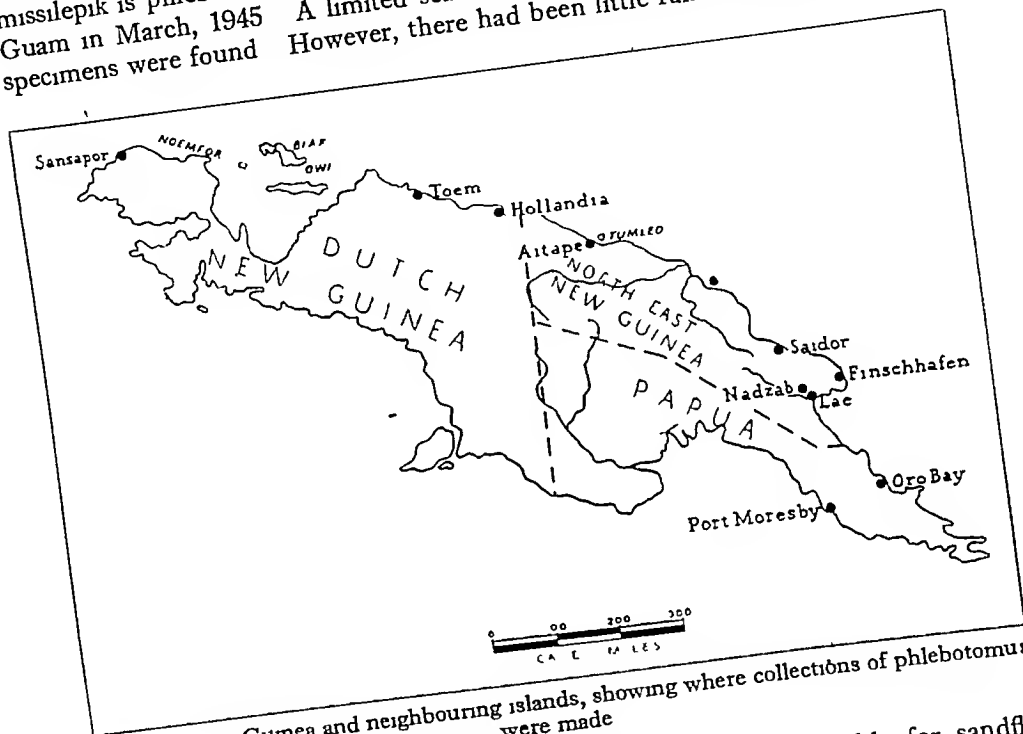


FIG 2 New Guinea and neighbouring islands, showing where collections of phlebotomus were made

if the genus does occur, conditions were possibly unfavourable for sandfly breeding Here it should be stated that, shortly after the first collections were made at Dobodura, Papua, in July, 1944, there was a dry period of almost a month By early September it was almost impossible to collect sandflies in jungle areas where in July and early August they had been numerous The presence or absence of these insects can only be determined by thorough searching throughout the year

The presence of *Phlebotomus* on Noemfor and Biak Islands, 60 miles apart, and about 50 and 90 miles respectively from the coast of New Guinea, points to the possibility of the occurrence of different species or races on land areas that are separated by water Since sandflies are not strong or high fliers, their presence on islands far removed from large land masses offers a subject for

speculation. Extensive collections in the Pacific and a careful study of specimens may eventually yield information that is highly significant with regard to the evolution of races or species of an insect.

### SUMMARY

Species of *Phlebotomus* as yet undescribed, were collected at widely separated Army bases in New Guinea and on islands many miles from the coast of New Guinea. The role played by these sandflies in the transmission of disease was not determined. A rapid method for collecting sandflies that involves use of an insecticide dispenser is described.

### REFERENCES

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HILL, G. F. (1923). *Bull. ent. Res.*, 14, 83.  
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## CORRESPONDENCE.

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### ACRIFLAVINE IN THE TREATMENT OF SCHISTOSOMIASIS

*To the Editor, Transactions of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In these Transactions (Vol 28, No 3, p 277) A C FISHER described the use of acriflavine in the treatment of intestinal schistosomiasis in the Stanleyville district of the Belgian Congo. He treated forty nine cases with acriflavine and found that in forty three cases, examination at the completion of his 5-day course showed only degenerate ova in the stools. After several months he found that out of thirty four cases he was able to follow up seventeen cases had become negative.

Recently, in Salisbury, Southern Rhodesia, with the assistance of Dr D M BLAIR and the permission of the Medical Director, seven patients were treated with acriflavine, who were suffering from *Schistosoma haematobium* and *S mansoni* infections. Four cases had *S haematobium*, and three *S haematobium* and *S mansoni* infections. These cases were adolescent school children and ranged in weight from 36.5 kg to 65 kg. They were all examined prior to being treated and found to be passing viable eggs. They were given the same dose as FISHER used, namely a total dose of 0.01 gramme per kg body weight given as five equal daily doses of 2 per cent acriflavine. They received this dose each day at 3 p.m., 3 hours after their midday meal. During the course of treatment none complained of any ill effects and they appeared to have no toxic symptoms.

The urine and faeces of these patients were examined immediately after cessation of treatment, and for 2 months after treatment had ended, at monthly intervals. At the end of treatment one of the cases with *S haematobium*

infection had ceased to pass viable eggs at 1 month after treatment the same patient remained negative and one patient with an *S. mansoni* infection was now negative at 2 months after treatment the patient with the *haematobium* infection remained negative but the case with the *mansoni* infection had begun to show eggs in the faeces again.

Although these cases could not be followed for longer than 2 months these findings differ from those reported by FISHER in that most of his cases became negative immediately after treatment. He was using cases of intestinal schistosomiasis as he had no cases of vesical schistosomiasis under his care. In this small series where every case had vesical schistosomiasis the effect of acriflavine was found to be almost negligible only one case remaining cured after 2 months.

I am, etc.,

National Institute for Medical Research,  
Hampstead, N W3  
8th December 1947

W F Rosa.

# ANNOUNCEMENTS.

## NEXT MEETING OF THE SOCIETY

The next meeting of the Society will be held at Manson House, 26, Portland Place, London, at 7 30 p m on Thursday, 15th April, 1948. A paper on "Diseases of tropical origin as seen in captive wild animals" will be read by Dr R R E REWELL, of the Zoological Society of London

## MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are temporarily in the British Isles. Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

AWOLUYI, S O, Nigeria  
 AL-ABED, H E, Iraq  
 ALEXANDER, G L, Gold Coast  
 CAFFREY, P J, Nigeria  
 CHARTRES, J C, Nigeria  
 CHWATT, L J, Nigeria  
 CLARK, MILDRED, Natal  
 CUMMINS, Lt-Col P H, India  
 DAVIDSON, Lt-Col T J, India  
 DAVIES, C W, Kenya  
 DAVIS, D H S, South Africa  
 DICK, G W R, Uganda  
 DIXON, P, Belgian Congo  
 DUGGAN, A J, Nigeria  
 EGWUATU, O S, Nigeria  
 ERB, J T, Nigeria  
 FIELD, J W, Malaya  
 FOY, HENRI, Greece  
 GRAHAM-CUMMING, G, Hongkong  
 HARKNESS, J W P, Nigeria  
 HUGHES, M H, Gold Coast  
 HUNTER, W, Nigeria  
 IP, YEE Hongkong  
 JONES, S A, Palestine  
 KELSEY, H A, Nigeria  
 KENT, Lt-Col P W, India  
 KHAYATT, SAMI, Iraq  
 LINDSAY, Lt-Col D K LL, India

McCoy, O R, France  
 McLEISH, A C, India  
 MCPHERSON, D R, Malaya  
 MAGUIRE, E H C, India  
 MORTON, T A, Gold Coast  
 NELSON, J W, Northern Rhodesia  
 PAL, RAJINDAR, India.  
 PHILLIPS, C M, N Rhodesia  
 QUANTRILL, D W, Nigeria  
 RITCHIE, G L, Tanganyika  
 RUSSELL, S F, Assam  
 SAUNDERS, G F T, Gold Coast  
 SCRIMGEOUR, H, Singapore  
 SIMPSON, T, Nigeria  
 SMITH, CONSTANCE B, Malaya  
 SMITH, Lt-Col M C L, India  
 To, S SHIU-YUEN, Hongkong  
 THOMSON, F ADAM, Singapore  
 UTTLEY, K H, Hongkong  
 WATERMAN, J, Trinidad  
 WATT, Lt-Col GEORGE, Gold Coast  
 WHITE, T H, Tanganyika  
 WIGAN, W C, Nyasaland  
 WILKINS, E G, India  
 WILLIAMS, W R, Gold Coast  
 WILSON, C J, Kenya  
 WILSON, W A, Uganda  
 WING, W M, U S A



## NEW FELLOWS

At the meeting of the Society held at Manson House on 19th February 1948 the following twelve candidates were elected Fellow of the Society —

BARJANO, J F R. (MEXICO), Buenos Aires  
 EBB, J THOMAS, M.D. (TORONTO), M.R.P.S. (ALTA) Nigeria.  
 FALCONER, DOR I B., M.R.C.S., L.R.C.P. F.R.C.S.E., Nigeria.  
 FYVIE, W R., M.B., CH.B. (EDIN.), D.P.H. (WITWATERBRAND), S. Africa.  
 GAULDIE, R D M.B., CH.B. (CAPE TOWN), D.P.H. (WITWATERBRAND) S Africa.  
 KONTI, ATRICHA, M.D. (ATHENS), Greece  
 LEMOYNE, ERWIN M.D. (TIENTSIN) China.  
 MONTAGUE, ROGER, M.B. B.S. (LOND) Squadron Leader, R.A.F.  
 ORANGE, F C., M.B., B.CH. (WITWATERBRAND), Medical Inspector Malaria, S Africa.  
 PEACOCK, P N B M.B. CH.B. D.P.H. (CAPE TOWN) Medical Inspector Malaria, S. Africa.  
 STEWARD IAN B M.B., CH.B. (LEEDS) Iren.  
 WEST G F O.B.E M.B. B.CH., B.A.O. (DUBLIN), D.T.M. & S. (KNO), Malaya.

At the meeting of the Society held at The Royal Army Medical College Millbank, S.W. 1 on 19th March, 1948 the following twenty-seven candidates were elected Fellow of the Society —

ABDEL-MEMED, SAUTEL R., L.R.C.P. & S. (EDIN) L.R.C.P. & S. (GLAS) Egypt.  
 ANDERSON J C., M.D. (PARAGUAY) Paraguay  
 CHANCO, PEDRO P M.D. (PHILIPPINES), Philippines.  
 CHAN, GON HOK, L.M. (SINGAPORE) Hongkong.  
 DECANO, GUILLERMO M.D. (MEXICO), U.S.A.  
 DIEGUEZ, LUIS A., M.D. (LIMA) Peru  
 FARMAN FARMAIAN S M.D. (TEHRAN), Iran.  
 FRAYNORTH, ERIC ALBERT M.D. L.M.S.B., South Africa.  
 GRAY, J H M.B. B.CH. CH.B., D.T.M. & S. (WITWATERBRAND) South Africa.  
 GHOSH, Major B M.B. (CALCUTTA) I.A.M.C.  
 GONZALEZ, DINGO B M.D. (MEXICO) Mexico  
 HAYES, G T M O.B.E M.C. M.B. B.CH. B.A.O. I.M.S. (N), Kenya.  
 ILICICU CONSTANTIN R M. M.D. (MOSCOW) R.S.S.F.  
 JULIANO SERAFIN J M.D. (PHILIPPINES) Philippines  
 KILLOUGH JOHN H. M.D. (JOHN HOPKINS) D. (YALE) U.S.A.  
 MACLAUGHLIN, JOHN DENYS, B. (ALTIMORE) ASUT (ST LOUIS) Liberia.  
 MACLEOD R C., M.B., CH.B., D.P.H. (GLAS) D.T.M. & S. (KNO) Scotland.  
 MATHUR, P N., L.M.P. (MADRAS) India.  
 RAM, JAGAT M.B., B.S. (RANGOON), Burma  
 RAYNO, A. S. M.D. (SANCTO) Iraq  
 RAEID, S A., D.S.M. (SUDDAN), D.T.M. & S. (LIV), Sudan  
 RINGOLD, E. J. B.S., M.D. (TENTON) Liberia.  
 SCOTT DVID M.B. B.CH. (CANB) D.T.M. & S. (LIV) Gold Coast  
 SMITH, ROBERT LESLIE, M.D. (ILLINOIS), U.S.A.  
 TOPETE, FERNANDO, M.D. (MEXICO) Mexico  
 URS, M. B R., B.S., M.B., B.S. (MADRAS), Bangalore  
 WILSON, J MICHAEL, M.B. B.S. (LOND), M.R.C.P. (LOND.) England

## ADMISSION TO FELLOWSHIP OF THE SOCIETY

All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society

Anyone desiring to become a candidate for Fellowship of the Society should use the special form of application at the end of this journal

The annual subscription payable by Fellows is one and a half guineas (£1 11s 6d) which becomes due in advance on the 1st of April of each year

The TRANSACTIONS and the current YEAR BOOK of the Society are posted regularly to every Fellow whose subscription is not in arrear

Further information may be obtained from the Hon Secretaries, Manson House, 26, Portland Place, London, W 1, or from the Local Secretary of the district

## NOTICE TO FELLOWS

As a copy of each number of the TRANSACTIONS is posted to every Fellow whose subscription is not in arrear, Fellows are particularly requested to notify the Secretaries of any change in the address to which their TRANSACTIONS are to be posted.

When copies of the TRANSACTIONS are returned by the Post Office marked "Gone Away," "No Service," or "Insufficient Address," no more copies will be posted to that address but they will be retained at Manson House until further instructions are received

## PRIZE TO BE AWARDED IN 1948

### THE CONSULTANTS PRIZE

The Consultants to the War Office and the Armies in the Field in the late war, have presented a sum of money to the R A M C in order to found a Consultants Prize, to be competed for at intervals of 1 to 3 years

This prize will be awarded for the first time in 1948 and will be to the value of 25 guineas. The prize is open to serving officers of the Royal Army Medical Corps, holding a regular or a short-service commission

The first prize will be awarded for an essay of not more than 10,000 words on a professional subject, based on the author's own experiences between 1939 and 1946. It is hoped that these essays will ensure that valuable war experience which would otherwise be lost, will be recorded for future guidance and possibly for publication

Entries should be sent in through the usual channels, so as to reach the Hon Secretary, R A M C Prize Funds Committee, R A M College, Millbank, London, S W 1 not later than 1st August, 1948

## LIBRARY NOTICES

From the Society's file of the *International Journal of Leprosy*, Volumes 8 & 9 (1941, 1942) are missing and are not obtainable from the publishers the original stock having been destroyed in Munich

Of the *Annales de la Société belge de Médecine tropicale*, No 1 of Volume 19 (1939) is missing

Of the *Chinese Medical Journal*, Volumes 62 & 63 (1944, 1945) are incomplete

Of the *Journal of Tropical Medicine & Hygiene* No 4 of Volume 49 (1947) is missing

Gifts of any of the missing volumes or numbers will be gratefully received at Manson House

## NEW BOOKS RECEIVED

*Contributions from the Biological Laboratories in Princeton University* Vols. 14 and 15 Princeton University Press.

*History of Scottish Medicine* (in two vols.). By JAMES D. COVENE. London: The Wellcome Historical Medical Museum.

*Tuberculosis in the West Indies* by W. SUTTON GILMOUR. London: National Association for the Prevention of Tuberculosis.

## WAR DAMAGED LIBRARIES POST WAR RESTORATION

Fellows will be rendering service to the Society if they return to Manson House any numbers of the TRANSACTIONS which they do not wish to keep—particularly any numbers of Volume 40.

The Council wishes to thank those Fellows who have already responded by returning copies of the TRANSACTIONS.

## PHOTOSTAT SERVICE.

To help Fellow of the Society who cannot readily obtain access to medical periodicals and journals, arrangements have been made to supply Photostat copies of papers required.

By courtesy of the Dean and Council of the London School of Hygiene and Tropical Medicine the Library of the School has been placed at our disposal for this purpose. Copies will be made by Photostat Limited.

Fellows will be asked to pay only the actual cost of preparing the Photostat copy. The minimum charge is 1s. per page copied (see 6½ × 11½) but price list will be sent on request.

Application should be made to the Secretary Royal Society of Tropical Medicine and Hygiene Manson House, 26 Portland Place W 1 giving the name of the journal, volume and page number of the article required. Complete papers or specified portion of paper can be supplied, but in the latter case lucid instructions must be given.

## FOURTH INTERNATIONAL CONGRESS ON TROPICAL MEDICINE AND MALARIA

These Congresses will be held in Washington U.S.A. from 10th to 18th May, 1948. Fellows attending to be present are invited to notify the Secretary at Manson House 26 Portland Place, London, W 1.

For further particulars, see these TRANSACTIONS, Vol. 41 No. 1 page xv.

The meeting of the Society announced for 20th May 1948 at Manson House has been cancelled.

## SCIENTIFIC INFORMATION CONFERENCE.

The Royal Society has arranged, from 1st June to 2nd July 1948, Scientific Information Conference in London.

The Conference will be attended by representatives of countries providing information services in English, namely the countries of the British Commonwealth and the United States of America.

The Sections and Editors-in-chief are as follows—

Section 1 Publication and distribution of papers reporting original work.

Professor J. D. BURNAL, M.A.

Section 2 Abstracting Services. Sir D. V. CRADWICK.

Section 3 Indexing and other library services. D. J. E. HOLSTROM.

Section 4 Reviews, Annual Reports, etc. Professor H. MENDEL FOX, M.A.

Application for tickets of admission should be made in writing before 1st June to the Assistant Secretary The Royal Society Burlington House London, W 1 from whom further particulars of the Conference may also be obtained.

# Royal Society of Tropical Medicine and Hygiene.

TELEGRAMS ANTHELES LONDON  
TELEPHONE LANGHAM 2127



ZONAE TORRDAE TUTAMEN  
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MANSON HOUSE,  
26, PORTLAND PLACE,  
LONDON, W 1

Name in Full (in Block Capitals)		Titles, Qualifications, etc., and their Source	Home Address *	Address Abroad *	Number on Register of Fellows
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APPLICATION FOR ADMISSION AS FELLOW OF THE SOCIETY

\* When both addresses are given the address to which it is desired that communications be sent should be underlined

## SECONDER

Signature  
of Fellow

2

Signature  
of Fellow

2

PROPOSER

1 Extracts from Laws of the Society No 8 — "Either the proposer or the seconder must have personal knowledge of the candidate and vouch for him as in every respect suitable for election as a Fellow of the Society" No 24 — "Every Fellow shall pay an Annual Subscription of One-and-a-half Guineas (£1 11s 6d.)" No 25 — "The name of a newly elected Fellow shall not be placed on the Register of Fellows nor shall he be entitled to any of the privileges of Fellowship until after his first annual subscription (£1 11s 6d.) or composition fee (£23 12s 6d.) shall have been paid"

DECLARATION BY CANDIDATE.  
I hereby undertake, if elected, to observe and obey the Laws and Regulations of the Royal Society of Tropical Medicine and Hygiene, and to endeavour to promote its honour and interests

Signature of Candidate

19

FOR OFFICE USE	Application received	Approved by Council	Elected	First Subscrip- tion received
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*Back Numbers* Owing to the greatly increased demand for complete sets of the TRANSACTIONS, the stock of certain numbers had been exhausted These have now been reprinted, and any number can be supplied

### YEAR BOOK, 1948

List of Fellows (with Addresses) Alphabetically and Geographically arranged. The Society's Annual Reports, Laws, and other matter  
Price Five Shillings, *post free*

### MONOGRAPH

Monograph I (September, 1936) "Boomerang Leg and Yaws in Australian Aborigines," by C J HACKETT 66 pages, 17 pages plates, stiff board cover, linen back  
Price 5s, *post free*

### "MANSON CENTENARY"

Proceedings of meeting at Manson House on 12th December, 1944, with coloured portrait, price Two Shillings and Sixpence, *post free*

### "THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE"

An illustrated pamphlet by "Onlooker," describing the work and functions of the Society and the amenities of Manson House  
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The TRANSACTIONS may also be ordered through Messrs H K Lewis and Co, Ltd, 136, Gower Street, London, WC 1, or any other bookseller

### ADVERTISEMENTS

Approved Announcements are accepted by the Council for insertion in the TRANSACTIONS, due to be published in alternate months as follows —

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No 3	November 25th	No 6	May 25th

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# EDITORIAL NOTICES

Papers submitted for publication in these TRANSACTIONS should be sent to the Hon. Secretaries Mansion House 26, Portland Place London W.1

The submission of matter for publication will be understood to imply that it is offered to this journal alone.

If accepted for publication, the copyright of papers becomes the property of the Society but they may be re-published by permission of the Council, provided due acknowledgment be made of their having appeared in the TRANSACTIONS.

Papers should, if possible, be typewritten; they should be concisely written with subject matter logically arranged and sub-divided; with references and abbreviations in the form described below and with indications of the position, in the text, of illustrations, tables, maps, etc.

Titles should be as brief as consistent with clarity and in many cases the value of paper is enhanced by short summary at the end.

Temperatures charts, graphs and drawings should be, if possible, in Indian ink on Bristol board; with detail and essential lettering large enough to be clearly legible after reduction if necessary (Write in pencil if lettering on drawing is to be set up and printed.)

Illustrations—if the number sent in is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the author's expense.

## REFERENCES.

In the text, the date of publication, in brackets, should follow the name of the author quoted thus:—

T. MASON (1879) is due this epoch-making discovery.

At the end of the paper list of References should be arranged in alphabetical order of authors surnames, and details given in the following order:—(1) Surname of author (2) Initials of author (3) Year of publication, in brackets (4) Title of article, avoiding arbitrary capitals. (The title of the article is sometimes omitted but each list of references should in this respect be consistent throughout—giving all titles, or omitting all). (5) Title of journal (6) Volume number (7) Page number *g*—MASON P (1879). On the development of *Plasmodium falciparum* and on the merozoites considered as *sporozoites*. *J. Linn. Soc. (Zool.)*, 14, 201.

In the case of reference to book (1), (2) and (3) as above (4) Title of book (5) Edition and for volume, if more than one (6) Page number (7) Town of publication (8) Publisher's name, *g*—MASON, P (1905). *Tropical Diseases* 1st Ed., 447 London Cassell & Co., Ltd.

Reference to an Annual Report SWAZILAND (1937). *Annual Medical & Sanitary Report 1936* p. 16

Note The year of publication is not usually the year covered by the Report.

## ABBREVIATIONS.

The abbreviations used are those shown in the World List of Scientific Periodicals, 1934, which conforms to the rules of the International Code of Abbreviations for Titles of Periodicals Paris, 1930. In general nouns have capital, adjectives small, initial letters; articles, conjunctions and prepositions are omitted the place of accent is added only when uncertainty might arise, *eg*

<i>Amor</i> J. Hyg.	C. R. Acad. Sci., Paris.	J. Pharmacol.
<i>Ann. trop. Med. Parasit.</i>	C. R. Acad. Sci., Johannesburg.	Ned. Tijdschr. Geneesk.
<i>Arch. Schiffs- u. Tropenhyg.</i>	Deutsches Wasser	Trans. R. Soc. trop. Med. Hyg.
<i>Bull. Soc. Path. exot.</i>	Indian and Gas	Z. Hyg. Infektkr.

The following contractions are in use whether the number to be expressed is 1 or more.

<i>g</i> 1 cc 15g, 45 kg.)—	kilogramme kg.	millilitre, ml.
centigramme cg.	kilometre km	millimetre, mm.
centimetre, cm.	micron, $\mu$ .	ounce, oz.
cubic centimetre, c.c.	milligramme, mg.	pound, lb.
cubic millimetre c.mm.		

In order to avoid dangerous error in dosage "gram" and "gramme" are printed in full.

## REPRINTS AND BLOCKS.

Authors of Papers published in the TRANSACTIONS receive 25 reprints free of charge. Any additional number can be supplied on payment, provided application be made when submitting the Paper.

Blocks made for illustrations may be purchased by the author at half cost price.

# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

May, 1948

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No 6

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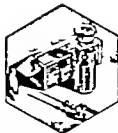
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VOL 41 No 6 MAY, 1948

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,  
on

Thursday, 19th February, 1948, at 7 30 p m

THE PRESIDENT,  
Sir PHILIP MANSON-BAHR, C M G, D S O, M D, F R C P,  
in the Chair

PAPERS

PATHOLOGICAL PROCESSES IN MALARIA

BY

BRIAN MAEGRAITH

*Professor of Tropical Medicine, Dean of the Liverpool School of Tropical Medicine*

I am going to try tonight to give you an overall picture of malaria as I see it, and point out some of the principal physiological and pathological processes which I believe influence the pathogenesis of the disease. Some of you may no doubt take the view that the time has not yet come for attempting to synthesize the little information we have acquired. You may consider that our knowledge is so limited that collection of further facts is of primary importance, rather than attempted integration of those we possess. This may be so but I believe that it is a good thing to pause every now and again to look at the material available and try to estimate its significance, and perhaps to formulate some hypothesis which can be tried out experimentally and decided one way or the other.

The processes which determine the reaction of the animal body to invasion by the malaria parasite are of both general and specific nature. Certain general processes, such as anoxaemia, are common to all tissue reactions. Others are more specific to the particular organ under consideration.

The basic phenomena concerned in the evolution of malarial tissue changes are of two kinds, e.g. those which progress more or less continuously throughout the disease, and those which are initiated by the schizogony of the parasite, and occur intermittently. Of the former the most important are anoxaemia and certain vascular and circulatory disturbances. The latter of course are the phenomena associated with the appearance of peroxysms or their equivalent.

Anoxaemia in malaria arises from pulmonary factors and from factors directly affecting the erythrocytes.

Changes in pulmonary ventilation and blood circulation occur in all forms of severe malaria: intense dilatation and hyperaemia of the septal capillaries, and a general slowing of the pulmonary circulation through the dilated engorged vessels often seriously impair the oxygenation of the circulating erythrocytes (SMITZ, 1946).

Of greater general significance, however, is the notable reduction of erythrocyte numbers brought about by the destruction of parasitized cells at sporulation, and the concurrent lysis of parasitized and unparasitized cells alike. The oxygen-carrying capacity of the blood is reduced in proportion and in certain circumstances the degree of anoxaemia so produced may be sufficiently severe to dominate the pathological picture and cause death. Such anoxaemia resulting from erythrocyte destruction alone has, for instance, been considered the cause of death in pigeons infected with *Plasmodium relictum* (HILL, 1942). Indirectly such factors as the inhibition of the escape of reticulocytes from the bone marrow during clinically active malaria, have also a decided effect on the degree of anoxaemia developed, since they influence the development of anaemia (TROWARD-NEUMANN, 1944; GRANICCA, 1945).

The invasion of extant erythrocytes in a given case exaggerates the prevailing anoxaemia, since in the invaded cell the effective concentration of haemoglobin is reduced by its conversion into haemozoin and the oxygen is removed from the oxyhaemoglobin by the actively metabolizing parasite (GHOSH and SERRIN, 1943; CHRISTOPHER and FULTON, 1938; ANDERSON and MORRISON, 1942). A further reduction in the effective oxygen-carrying powers of the invaded cells, and possibly of all erythrocytes, may result from changes in the physico-chemical properties of haemoglobin. The efficiency of erythrocytes as oxygen-carriers is further reduced in the late stages of malaria by intravascular agglutination, such as has been observed by KNISTLY and his colleagues during the course of malaria in transilluminated tissue of man, monkeys and bird (KNISTLY, 1941, 1943; LACK, 1947). The effect of this intravascular agglutination, which apparently is non-specific and results from

generalized anoxia, is the appearance of small clumps of cells which cohere loosely in the form of what KNISELY calls "sludge." This sludge circulates in the general blood stream and passes with difficulty through the finer vessels, so interfering with local blood flow and the oxygen carriage to the corresponding tissue. The individual cells comprising the sludge are incompletely oxygenated in their passage through the lung capillaries.

It will be seen that there are many factors acting in malaria tending to produce a condition of generalized anoxaemia. This condition in itself must give rise to some degree of tissue anoxia which may be sufficient to bring about cellular changes, but usually its effects are considerably enhanced by the development of general and local changes in blood flow, as described below.

#### GENERAL CIRCULATORY DISTURBANCES.

Certain changes in the circulation occur during the paroxysm. In the cold stage there is peripheral vasoconstriction associated with an abrupt rise of blood pressure and a generalized slowing of circulation time. As the hot stage develops, vasoconstriction gives place to peripheral dilatation. The blood pressure falls somewhat and circulation time increases together with cardiac output (MIKELADSE, 1923, ALTSCHULE, 1945). The vasoconstriction of the cold stage is not easy to explain, but in the hot stage the circulatory effects of fever appear. In a straightforward case of malaria undergoing regular paroxysms the hot stage lasts only a few hours at most, and the temperature subsequently becomes normal and with it the general circulation is readjusted. Sometimes, particularly in falciparum malaria, the temperature remains elevated, so that the metabolic rate is persistently raised, cardiac output remains high and a continuous strain is placed upon the heart. In spite of this the heart rarely fails as a pump, except where the changes in the heart muscle are sufficiently great to impair the function of the myocardium. More frequently, some degree of generalized vascular collapse supervenes. In its extreme form this is indistinguishable from medical shock (ATCHLEY, 1930).

When the body temperature remains persistently high or continues to rise, a vascular crisis may be precipitated, particularly if there is uncompensated loss of fluid, and under these circumstances medical shock may develop (KOPP and SOLOMON, 1937; HARTMAN, 1936, 1938). In these cases the basic pathological findings are those of acute circulatory failure (MOON, 1938), and not usually those associated with hyperthermia *per se* (MALAMUD, 1946). Phenomena of this sort occur in malaria associated with hypervæmia and, very occasionally, with the cold stage of the paroxysm, but the vascular disturbances of malaria are as a whole not usually associated with high temperatures. Profound circulatory changes are more frequently found in forms of pernicious malaria, often accompanied by low oral temperatures. Under these circumstances the appearance and physiological state of the patient are again identical

with those of severe shock. The skin is cold and moist, the pulse is rapid, thin and easily compressed, and the respiration superficial and irregular. The blood pressure is low and there is haemoconcentration and other evidence of acute reduction in blood volume. Many writers have tried to associate such syndromes with derangement of the adrenal glands but in individual cases evidence of this is frequently lacking (PARASKEAU and LEMAITRE, 1913; FLOCH, 1945). Ample evidence has been produced recently demonstrating that genuine shock, accompanied by loss of blood volume may occur in malaria, particularly in *P. falciparum* infections. Thus KEAX and SMITH (1944) reported that 22 of 100 fatal cases of *P. falciparum* malaria examined by them died from shock or related phenomena, and KEAX and TAYLOR (1946) recently described six cases of *P. falciparum* infection which developed symptoms of shock during the clinical activity of the disease. In these cases there was clear evidence of loss of circulating blood volume, indicated by haemoconcentration in the form of a rise of red blood cell count and haemoglobin concentration associated with gross reduction in blood pressure and clinical signs of medical shock. Similar cases have been reported by other workers.

The pathological findings in such patients are fundamentally similar to those seen in shock caused by other agents.

The general circulatory changes mentioned above and the appearance of shock must influence often profoundly the blood circulation through the tissues. It is equally true that the changes in blood flow so initiated in certain organs may physiologically affect still further the general circulation. For instance, reduction of flow through the adrenal or the development of a state of local anoxia may give rise to output of adrenalin, with its usual circulatory effect (VAN LIERE, 1942).

It might be pointed out here in parenthesis that such changes in the adrenal and other endocrine organs may also be largely responsible for the humoral phenomena upon which the theory of the anaphylactoid basis of the paroxysm has been based, as for example the rise of plasma potassium, the fall of sodium, and the changes in blood sugar.

#### LOCAL CIRCULATORY CHANGES

The circulation in the organs which has been mentioned, influenced by the general changes occurring in malaria, but certain local conditions modify these effects considerably. For instance, the anatomical arrangements of vessels within an organ, the presence of nerves and sinusoids all play a significant part in the local effects of general circulatory disturbances. Again, local vascular reflexes may be evoked. For instance the tissue of the renal cortex may become specifically anoxic following the shunting of the blood flow from the cortex to the medulla (MACGRAITH and FINDLAY 1944; MACGRAITH *et al.*, 1945; TRUETA *et al.* 1946). Reflex changes may also occur

in circulation through the lobules of the liver arising in certain cases from impedance of the escape of hepatic venous blood (MAEGRAITH, ANDREWS and GALL, 1947). Such local reflexes appear to be initiated either locally as a result of existing damage to the tissue, or as part of a general vascular disturbance. Once such changes have been established it is easy to see how they can modify the pathological changes which develop in a given organ.

Mechanical obstruction to blood flow may also be evident in some tissues. The vascular endothelium sometimes swells and occasionally exhibits phagocytic activity, in the late stages of the disease the endothelial cells often develop the property of "stickiness" to leucocytes, which tend to collect about the periphery of the vessels, causing some interference with the blood flow. In areas where collateral circulation is poor, such as the heart muscle and certain parts of the brain, accumulation of parasitized cells, free parasites, red cell debris and sometimes haemozoin may also give rise to some circulatory retardation. Intravascular agglutination of the red cells to form sludge, and sometimes true thrombosis may be concerned in the late stages of the disease. The degenerative changes going on in the cells of the tissue give rise in some organs to swelling of the parenchymal cells, which is sufficient under certain circumstances, e.g., sometimes in the liver (HIMSWORTH, 1947), to obstruct the blood flow through the small vessels.

These factors may give rise to some degree of mechanical obstruction to circulatory flow, but more important than all of them is the development of the physiological condition of vascular stasis, in which the erythrocytes impact to form an almost homogeneous mass and local circulation is temporarily suspended. This phenomenon is fundamentally reversible and results from local loss of fluid from the circulating blood (LANDIS, 1927 FLOREY, 1926). It develops most readily, I believe, in those organs in which the capillaries and small vessels are normally impermeable to protein. Thus stasis during the course of malaria is particularly well seen in the brain and the heart. In other organs in which there is some evidence that the small vessel walls may be normally permeable in some degree to protein, stasis very seldom appears. It is practically never seen in the liver, kidneys or the bone marrow. The importance of this phenomenon will become clear when we examine the development of the pathological changes in the brain. It will be evident at this point however, that the stased vessel is temporarily completely closed to the circuit of blood, so that the tissues in its immediate neighbourhood, and those which depend upon it for oxygen, will, for the time being be practically anoxic.

It is interesting to speculate on the initiating factor which starts off the circulatory phenomena of malaria. Those occurring during the paroxysm can probably be most easily explained on the basis of some form of stimulation and possibly concomitant inhibition of certain vasomotor centres, particularly those in the hypothalamus resulting from the release of some diffusible substance at the time of sporulation of the parasite. Similar central factors may

control the establishment of the state of vascular collapse associated with acute reduction in circulating blood volume. In certain cases there may be considerable direct loss of body fluid from vomiting, sweating and diarrhoea, but in the majority of patients such loss is inconsiderable in relation to the total reduction of blood volume. The immediate cause of this sudden reduction in blood volume is obscure. The degree of parasitaemia is not the deciding factor since shock often appears in cases in which there is only moderately severe invasion of the erythrocytes. Anaemia is also not the precipitating factor although it probably plays an important part in cases in which the lysis is heavy and rapid (KILAM and TAYLOR, 1946). Some other factor must be at work. The similarity between the vascular phenomena of malaria and those of general acute inflammation is sufficiently striking to suggest the involvement of some common basic mechanism. This view is supported, as we shall see by the development of endothelial changes in malaria.

### TISSUE ANOXIA

In addition to the sudden loss of circulating blood volume which occurs in malarial shock local loss of fluid through the vascular endothelium is evident in many tissues. In severe malaria, endothelial damage may be detected histologically and is indicated physiologically by the appearance of stasis, particularly in the brain. What produces these changes in vascular endothelium is not clear but a general factor of great importance is undoubtedly the production of tissue anoxia, which exists potentially in all cases, because of the prevailing anaemia, and general vascular and circulatory disturbances. These factors may all have a profound effect on the oxygen supply to the individual cells of the tissues, and some degree of tissue anoxia is inevitable, and may be sufficient to lead to degeneration and ultimately necrosis.

The reduction of blood flow achieved by the various mechanisms described above leads to the production of stagnant and other forms of anoxia which in turn damage the endothelium, and further increase its permeability with consequent escape of protein and fluid into the tissues. In organs such as the brain this process is progressive and eventually the cells go into stasis and pack together as masses which may obstruct the blood flow completely giving rise to anoxia of the surrounding tissue and finally to the degenerate changes and necrosis. The ultimate lesion formed is dependent upon the circulatory arrangements of the organ concerned, but anoxia appears to play a part in the pathogenesis of all tissue changes.

I think changes in the permeability of the vascular endothelium are of supreme importance in the development of lesions in certain organs. We unfortunately have no information regarding the action of the parasite or its products in this connection. The identification of some substance capable of initiating such endothelial changes would I think, make the whole conception of malaria more intelligible. The elaboration of some diffusible





dependent to a considerable extent on the nutritional state of the host and the presence or absence of certain essential substances such as biotin and pantothenate. The proper protein balance of the body and presence of certain essential amino acids are also of extreme importance.

The epidemiological features of immunity are outside the scope of this discussion. The immune reactions of the host are of importance here in so far as they affect the cellular response of the tissues to the presence of parasites and the various associated blood vascular phenomena we have discussed. The splendid work of TALLAFERRO and his colleagues has helped greatly in the general understanding of the part acquired immunity plays in the cellular reaction of the host in the disease and such things as the specific stepping up of phagocytosis in the spleen, liver and bone marrow must be considered in any attempt to synthesize the ultimate effect of the disease processes on the tissues. The suggestion of GEAR (1946) that autoantigens may be produced during malarial infections should also not be overlooked.

#### PATHOGENESIS OF LESIONS IN THE BRAIN AND LIVER

I propose now to show how some of the general processes defined above enter into the development of lesions in special organs. We shall have time to deal with no more than the lesions of the brain and to a limited extent the liver.

Finally as a matter of general interest and in order to stress what was said earlier about the value of occasional synthesis of available material as a method of finding a lead for experimental work, Dr. AXDAKE will give you an account of how we are tackling some of the more concrete problems in the pathogenesis of the liver lesion.

#### LESION IN THE BRAIN

##### *The Histological Picture*

The most evident features of the histological changes in the brain in severe malaria are the dilatation and congestion of the small blood vessels and the concomitant haemorrhages. In most cases the small blood vessels are deeply congested and the contained erythrocytes are frequently heavily parasitized. The larger vessels are equally congested but in them the parasitized cells are apparently concentrated on the periphery of the blood stream and line the endothelium, the erythrocytes in the centre of the vessel being often unparasitized. In some specimens parasites may be very few even in the small vessels. Many authors have described vessels in which the erythrocytes are so closely packed that they are practically "fused" into homogeneous masses in which the individual cell cannot be identified.

Evidence of endothelial damage is common: there are for instance frequent small perivascular extravasation of erythrocytes similar to the lesions



explain the ordinary clinical findings in malarial cases, since as DUNCAN and CLARKE (1917) pointed out the effect of specific therapy clearly indicates that the lesions in the brain must largely be of a reversible and temporary nature. After reviewing the literature, REEDER (1944) has recently concluded that thrombosis and embolism were uncommon in malaria.

Mechanical interference with blood flow may occur to a limited extent in the malarial brain. It is conceivable to any degree, however only in the presence of very heavily parasitized blood, except, perhaps, in cases in which intravascular sludge formation is extensive. There is general agreement that the influence of endothelial swelling and degeneration on the blood flow must be regarded as minimal.

It is thus clear that some other process which is rapidly reversible must be responsible for the apparent impedance of the blood flow in the brain.

GASKILL and MILLAR (1920) considered the significant circulatory change in malaria to be the frequent haemorrhages which occur in association with the vessels, rather than changes of intravascular flow. This is probably correct so far as the occasional persistent cerebral lesions of malaria are concerned, but it is difficult to see how the common signs of malarial involvement of the brain can be related to anything else than temporary and reversible disturbances in the blood flow through the small blood vessels.

Intravascular stasis or near stasis (in the sense of almost complete occlusion of a vessel) seems to be the process which can most readily explain both the clinical and pathological findings.

As we have mentioned above this process depends for its development on the escape of abnormal amounts of protein and fluid across the endothelial cell membrane into the surrounding tissue, so that a local reduction in circulating fluid volume occurs, and the erythrocytes become compressed into what FLOREY (1926) calls a "transparent mass of corpuscles". The circulation in the affected vessel is stopped, and will not resume until the stasis is resolved. There is no true agglutination or clotting of the involved corpuscles and the process is primarily reversible. With the resolution of stasis, the physiological impermeability of the vessel walls to protein is restored and fluid return to the lumen and circulation is re-established.

It is possible to understand the temporary interference with blood flow in such circumstances, and the consequent tissue damage, including that presenting in the endothelium itself.

The histological evidence of the existence of stasis in the brain vessels is good. For instance, although some small degree of true thrombosis can be found histological examination of brain tissue discloses little or no fibrin in the majority of the apparently occluded vessels. Again, many authors have noted the appearance of homogeneous masses of corpuscles in the vessels and the frequent difficulty in identifying individual erythrocytes.

BRIAN MAEGRAITH

From the point of view of the clinical reversibility of severe cerebral signs, stasis appears to me to be the only reasonable explanation of the occlusion of the cerebral circulation. LANDIS (1927) has shown that the prime factor in the evolution of such endothelial changes in permeability is local anoxia. It is necessary therefore to postulate the existence of some considerable degree of anoxia in the brain in severe malaria.

Many of the factors concerned in the development of anoxic states in the disease have already been referred to. What is difficult to assess in this case are the factors which render the vessels of the brain so susceptible to anoxia, in comparison, say, to those of the liver which seldom become stased.

The cerebral lesions of malaria are not specific to the disease. Similar changes are found in conditions of severe anaemia, hyperthermia, mechanical obstruction to the main vessels to the brain, and in narcotic poisoning, including poisoning by alcohol and barbiturates.

HARTMAN attributed the lesions of hyperthermia to shock and associated anoxia. He came to the conclusion that these lesions were so similar to those arising in the other conditions referred to in the preceding sentence, and in "cerebral" malaria, that it was reasonable to assume a basic pathogenic factor, which he considered to be anoxia. This view is supported by experimental work which has shown that the histological effects of induced anoxia on the central nervous system are essentially similar to those seen in malaria, and include apparent obstruction to the small vessels, haemorrhages about the vessels and circumscribed area of necrosis (VAN LIERE 1942, SCHMIDT, 1928, 1935).

The above will give some notion of the complicated effects of anoxia *per se* on the central nervous system. It is yet to be determined whether these effects are exaggerated in malaria by specific activities on the part of the plasmodium.

The possibility that some agent, other than the general factors previously referred to might specifically influence the brain tissues in malaria has been considered by many authors but, as we have seen nothing of this nature has yet been identified. ANDERSON, MORRISON and WILLIAMS (1942) and ANDERSON and MORRISON (1942) have excluded haemozoin as a possible toxic factor. They found that in animals generalized vasodilatation extravascular haemorrhages thrombosis and infarction resulted from injection intraperitoneally or intravenously of large doses of disodium ferrihaemate, but they concluded that the tissue lesions were secondary to circulatory and vascular disturbances and not the result of direct toxic action of the pigment, which was harmless in the form existing in the plasmodia. In their view, the tissue damage developed as the result of anaemia (anaemia) following vascular obstruction.

Although no specific agent has yet been identified there is general

evidence of the existence of some substance capable of provoking changes in the vascular endothelium in malaria. CANOON (1941) has pointed this out in comparing the lesions of malaria with those of generalized inflammation. It may well be that the reason why such an agent has not yet been identified is that it has been sought in the wrong place. The possibility also exists that the cells of both the endothelial lining of the vessels and the tissues may be damaged by histotoxic agents, which, by stabilizing oxytocochrome (as in the case of barbiturate poisoning) may interfere with cellular metabolism to a degree out of proportion to the prevailing lack of oxygen.

RICHDS (1944) has stated the case very clearly. He has pointed out, in agreement with HARTMAN that the cerebral lesions of malaria are non-specific, similar lesions occurring for example, in hyperthermia, in which anoxia is the determining factor. In his view anoxia thus most readily explains the appearance of cerebral lesions in malaria. This anoxia arises, as we have shown above, from a variety of causes, including anaemia, interference with oxygen-carrying properties of the parasitized erythrocytes, and circulatory disturbances initiated both by cardiac failure and vascular collapse. The lesions in the brain arise in the first instance from leakage of fluid through the vessel walls, following increased permeability engendered by local anoxia. Slowing of intravascular flow causes and ultimately causes. When the vessel wall is sufficiently damaged diapedesis of erythrocytes takes place giving rise first to extravascular extravasation and subsequently to haemorrhage into the tissue. The picture is complicated by various phenomena, such as the "stickiness" of parasitized cells to the endothelium and the effects of local accumulation of toxic metabolites. In the first instance, these changes are reversible, but become irreversible, and lead to permanent tissue damage, unless the anoxic conditions are rapidly relieved.

In the pathogenesis of the brain lesions of malaria, there is thus much to be said for the view that the basic process is the appearance of vascular stasis arising out of a condition of local anoxia and damage to the endothelium of the vessels.

What is most intriguing, however is the fact that similar changes are not evident in certain other organs, even in the same patient. It is a common experience for instance, to find evidence of stasis in the brain, and none in the liver. In fact any extensive evidence of vascular obstruction, other than congestion, is most uncommon in the latter organ. In the ordinary course of events, as MOON (1938) points out, stasis is to be expected once the vessel wall is damaged. It is unlikely that there is any specific damage peculiar to the vessels of the brain in malaria. On the contrary there is, as we have seen, general evidence of vascular damage in most tissues. Why then, is stasis found commonly in the brain and so seldom in the liver? I believe the answer lies in inherent differences in the properties of the endothelial cells

of the vessels in the various organs. There is some evidence to show that the small vessels of the brain are normally highly impermeable to protein, whereas the sinusoids of the liver normally allow the passage of certain amounts of protein, so that the liver cells are bathed in fluid with a high protein content (MOON, 1938, DRINKER and FIELD, 1933). Small changes in permeability to protein might therefore be conceived to effect considerable changes in cerebral circulation owing to increased local loss of circulating fluid, whereas similar changes in permeability would have minimal effects on the micro-circulation in the liver.

I think the lesions of the liver, as in the brain, can be explained best by postulating a state of local tissue anoxia, but the mechanism of the production of this anoxia is essentially different in the two organs. In the liver, the circulation is slowed not by stasis, but by local failure of flow, chiefly in the central regions of the lobules, arising primarily from obstruction to the escape of venous blood. The interference with venous outflow is, we believe, often reflex in origin, but, as HIMSWORTH (1947) has recently pointed out, similar effects, so far as the tissue is concerned, may also be occasioned by localized parenchymal swelling.

We have outlined the arguments for our views on the pathogenesis of malarial centrilobular changes elsewhere (MAEGRAITH *et al.*, 1947), and I now leave discussion of them to Dr ANDREWS.

(For References see page 702)

## PAPER

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### THE LIVER LESIONS IN MALARIA

BY

W H HORNER ANDREWS, B M, B CH (OXON),  
*Liverpool School of Tropical Medicine*

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Professor MAEGRAITH has just stressed the importance of local vascular phenomena and the reactions of individual organs in the pathogenesis of the lesions in malaria, and has suggested that in the brain the lesion is primarily due to stasis and its concomitant effects. He also pointed out that this phenomenon does not appear to be the underlying factor in the pathogenesis of lesions in the liver owing to the different physiological reactions of this organ.

I should like to add a brief account of our views on the development of hepatic lesions as an illustration of the effects of local changes in the vascular

flow leading to anoxic changes in the parenchymal cells. In this discussion I will exclude such factors as the hypertrophy of the reticulo-endothelial system except in so far as they may cause some vascular obstruction, and I will deal primarily with the pathogenesis of the degeneration and necrosis which may occur in the lobules.

### THE HISTOLOGICAL PICTURE

Liver changes in malaria are very variable in degree and may not always be present. When well established the histological picture is one of centrilobular degeneration and necrosis. The organ is congested the central vein and sinusoids are filled with erythrocytes, many of which contain parasites. The sinusoids and central vein may be dilated but empty when anaemia is severe. (See Figs. 1 and 2.)

The erythrocytes in the hepatic vessels retain their form and are not impacted as in the brain. There is little evidence of stasis or thrombosis in the blood vessels, whose endothelium, as Professor MACRAITH pointed out, usually shows few histological changes. KLOTZ (1929) found no evidence of mechanical obstruction of the blood flow in the liver and contrasts the picture with that seen in the brain. Obstruction of the sinusoids and liver blood vessels has, however, been described. Stasis, thrombosis, collections of pigmented macrophages, free parasites and pigment and agglutination of erythrocytes, some parasitized have been recorded but such obstruction is rare.

The Kupffer cells are often swollen and filled with erythrocytes, parasites and debris. They appear in places to obstruct the lumen of the sinusoids, and even distort the columns of parenchymal cells, but such gross hypertrophy is by no means invariably present. The parenchymal cells may show only mild acidophilic granular degenerative changes, but fatty degeneration is common and is most evident in the cells of the central zone of the lobule or when those cells are necrotic, in the cells at the periphery. More severe degeneration and necrosis is frequently seen in human and animal malaria, the changes being most marked at the centre of the lobule. In cases of falciparum infection and blackwater fever showing extreme hepatic necrosis the cells around the portal tracts may alone survive. (DUDGON and CLARKE 1919 GASKELL and MILLAR 1920 TALLAFERRO and CANNON 1936 TALLAFERRO and MULLIGAN 1937 REDON and STRATMAN THOMA 1942 KEAN and SMITH 1944.)

### PATHOGENESIS OF CENTRILOBULAR NECROSIS

The hepatic lesions in malaria resemble those found in a variety of conditions, notably right-sided heart failure and Chauri syndrome (CHAURI 1899 THOMPSON and TURNELL, 1912) which are associated with tissue anoxia arising from interference with the outflow of blood from the liver. Right-sided heart

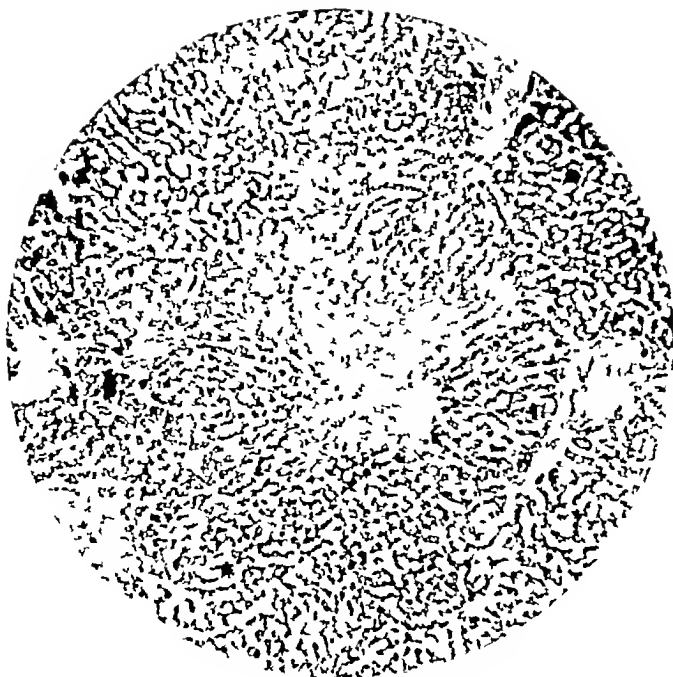


FIG 1—Sections from the liver of a patient dying from an acute falciparum infection. Centrilobular necrosis is well marked. The sinusoids appear dilated and congested.

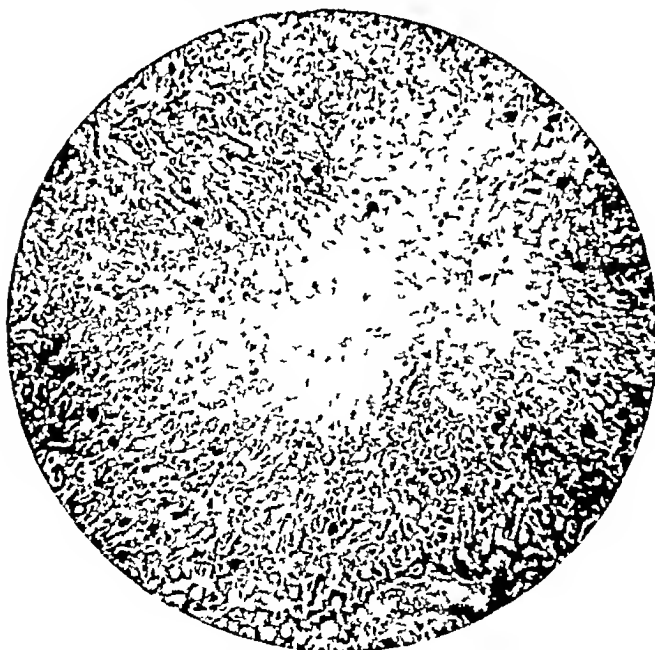
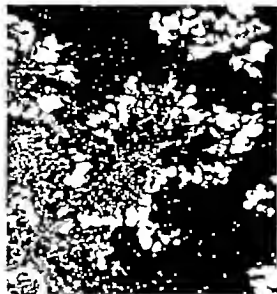


FIG 2—Section from the liver of a monkey dying in an acute exacerbation of chronic knowlesi infection. The appearance is very similar to that of Fig 1.





—Section from the liver of rat, 0.1 c.c. carbon tetrachloride per 100 gramme body having been administered subcutaneously 20 hours previously. Centrally there is hyaline and necrosis. In the mid zone there is hydropic necrosis. Peripherally the cells are more normal but swelling is evident.



4—Section from the liver of normal rat after injection of 1 c.c. Indian ink into scapular vein. Note the relatively large sinusoids.

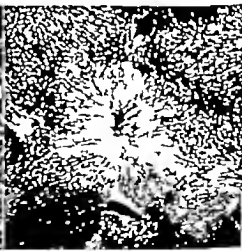


FIG 5—Section from the liver of rat 20 hours after subcutaneous injection of 0.1 c.c. of carbon tetrachloride per 100 gramme body weight. Indian ink was injected as previously. The calibre of the sinusoids is considerably reduced except peripherally.

failure may occasionally contribute towards the pathological changes in the liver (GASKELL and MILLAR, 1920), but in many cases there is no evidence of cardiac failure, and presumably therefore no rise in pressure in the inferior vena cava. On the contrary in many patients in whom the liver lesions are well developed death follows the development of a state of medical shock (ATCHLEY, 1930), in which condition right sided cardiac failure does not occur and the venous return to the heart is presumably diminished the pressure in the inferior vena cava being reduced (WIGGERS, 1918, MANN, 1919, RIDGON, 1942, MAEGRAITH, 1944).

Centrilobular necrosis can be readily produced experimentally. The mechanisms of its production fall broadly into two groups: those which reduce the oxygen content of the blood reaching the liver (RESNIK and KEEFER, 1925-6, ROSIN, 1928, RICH, 1930) and, what is virtually the same, those which slow or diminish the flow of blood through the liver. The latter effect may be caused by mechanical interference with the portal vein and hepatic artery (BAINBRIDGE and LEATHES, 1906-7, WHIPPET and HOOPER, 1916, ROUS and LARMOIRE, 1920 and BEHREND *et al*, 1922) or by impendence of the outflow of blood from the liver (BOLTON and BARNARD, 1931, SIMONDS and JERGSON, 1935, WEATHERFORD, 1935). All these conditions lead in greater or lesser degree, to interference with the oxygen supply to the cells. The cells of the central zone are anatomically the last to be supplied by the oxygenated blood entering the lobule. These cells are consequently especially exposed to the effects of any reduction in the oxygen content of the blood (MACCALLUM, 1928).

In many cases of vasomotor collapse the viscera especially the liver, are congested even in the absence of signs of right sided heart failure as has been emphasized by MOON (1938). Dogs shocked by anaphylaxis (BAUER *et al*, 1932, SIMONDS and BRANDS, 1927, WEATHERFORD, 1935) or by haemorrhage (WIGGERS *et al*, 1946) show an increased resistance to the passage of blood through the liver, the obstruction being localized in the hepatic veins. We have postulated that in man a similar resistance to the outflow of blood from the liver due to constriction of the smaller radicles of the hepatic vein might arise reflexly in cases of general vasomotor dysfunction. Such a constriction would lead to engorgement of the liver and a slowing of the hepatic blood flow consequently reducing the amount of available oxygen at the centre of the liver lobule. The evidence in favour of this hepatic "shunt" reflex has been discussed elsewhere (MAEGRAITH *et al*, 1947).

#### EXPERIMENTS

There is good evidence of the existence of reflex control of the hepatic venous tree in certain animals, notably the dog but not in all. It is thus extremely difficult in this particular instance to consider the results of animal experiments in terms of the human pathological findings. Ultimately evidence

must be obtained in man. Nevertheless, animal experiments are necessary for further general study of the liver and its vascular supply and for establishing techniques which may subsequently be used in human experiments.

The study of a problem of this sort seems with technical difficulties. We have for instance, spent a great deal of time in trying to find some method of obtaining a standard centrilobular necrosis. One way of approach has been the study of the action of well-established toxic agents including carbon tetrachloride. This work has already provided us with one salutary lesson, for in the development of lesions following the administration of this substance reflex control of the lobular blood flow if present appears to be of secondary importance, the essential factor being as HENSHAW has shown (1947), acute swelling of the hepatic polygonal cell (See Figs. 3, 4 and 5). The effect of carbon tetrachloride however depends considerably upon the dose administered. After large doses in animals swelling of the parenchyma is clearly a predominant feature. In man large doses give rise to a similar effect therapeutic dosage on the other hand sometimes leads to acute engorgement of the liver with associated centrilobular changes (CANNON and KAREKARATY, 1936). We have noticed similar changes in animals after small doses, and have interpreted these findings as evidence of activity other than cellular swelling. Here again we suggest interference with the intralobular blood flow is occurring. In any case the eventual changes in the parenchymal cells appear to result from anoxic local states. Whether basically dependent on swelling of the liver cells or arising from obstruction to venous outflow the fundamental process seems to be the impotence of the intralobular flow.

### CONCLUSION

We regard the lesions in the liver in malaria, like those in the brain, as an example of the effects of tissue anoxia. This basic factor of anoxia arises from certain general changes affecting all tissues, and from local conditions which are more or less specific to the individual organs. In estimating the pathogenic processes involved in the development of lesion in malaria it is therefore essential to consider not only the general factors but also the special physiological reaction of the organ concerned.

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## DEMONSTRATION

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### THE EXOERYTHROCYTIC PARASITES OF *PLASMODIUM CYNOMOLGI*

BY

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(PRESENTED BY DR P. C. C. GARNHAM)

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Pictures of the developmental stages of *Plasmodium cynomolgi* in the liver were shown. These were photomicrographs in colour of sections stained with Giemsa stain. The forms illustrated were 7-day-old schizonts, probably inside the parenchymatous cells, and measured about  $26\mu$  to  $30\mu$  in diameter.

There are a large number of nuclei scattered throughout the cytoplasm. Some of the bodies show a clear-cut vacuole, others, pseudopodium-like arms. A description of these pre-erythrocytic forms has been published recently (SHORTT, GARNHAM and MALAMOS, 1948), and it is unnecessary to say anything further here, except that these forms occur in monkeys which have been bitten by very large numbers of mosquitoes infected with *P. cynomolgi*. After biting, the mosquitoes were ground up in plasma saline and the suspension was inoculated intraperitoneally and intramuscularly into the same monkey, which must altogether have received a really enormous dose of sporozoites.

These schizonts might be compared with those of other haemosporidian parasites which develop in the liver. The largest of these is *Hepatocystes* (*Plasmodium*) *kochi*, and this also grows in the parenchyma cells, eventually forming merocysts up to 2 mm in diameter. At an early stage, the developing schizont of *H. kochi* is indistinguishable from the mature schizont of *P. cynomolgi*, except that it is larger (GARNHAM, 1947 and 1948). The exoerythrocytic schizonts of *P. gallinaceum* in the liver appear to develop chiefly in the Kupffer cells, the mature forms measuring about  $10\mu$ . Lastly, in bit malaria, as recently described by MERR and GOLDBLUM (1947), the unpigmented schizonts seem to be in the endothelial cells of the blood vessels, both sinusoids and larger veins. These are little more than  $6\mu$  in diameter. There is thus a range in size between the smallest bit parasites and those of avian malaria, to the considerably larger schizonts of *P. cynomolgi* and the macroscopic ones of *H. kochi*. The first two are found in various organs besides the liver, the last two appear to be confined to the parenchymatous cells of that organ alone.

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## DISCUSSION

The President Sir Philip Manson-Bahr The subject is now open for discussion. You have seen some very beautiful pictures, and had a lucid explanation of those changes that take place in the organs in malaria. It is an entirely new approach and as Professor MARGRAITH has said, it does us good to review the state of our knowledge on any subject from time to time. We are getting back to the good old days when people made hypotheses and proceeded to work on that basis. I hope you will first of all discuss together the papers of Professor MARGRAITH and Dr. ANDREWS, and then go on to the erythrocytic cycle.

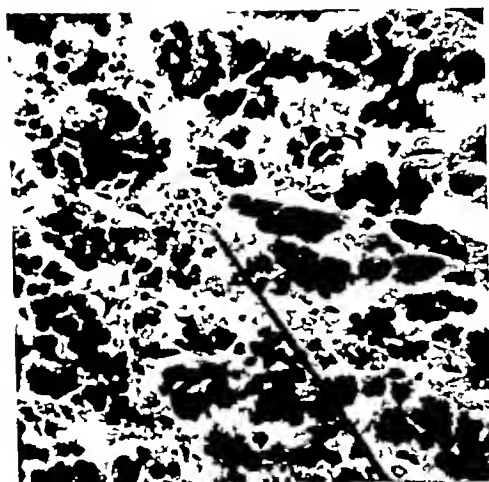
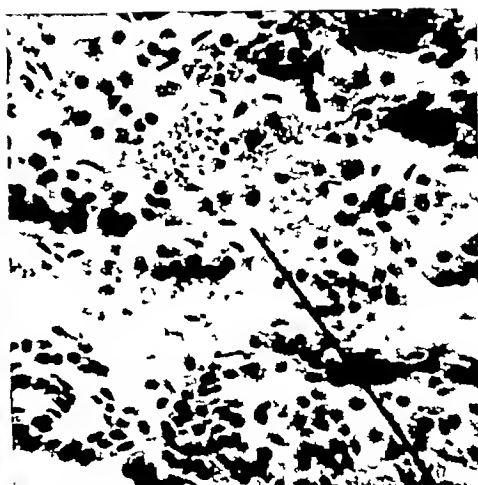
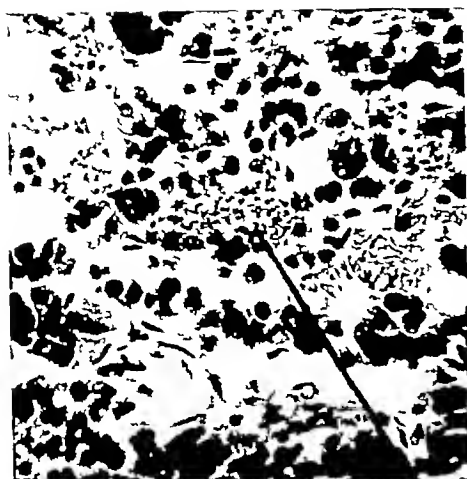
Lieut.-Colonel A. Meneses I should like to ask Professor MARGRAITH whether the suprarenal glands were examined in the cases exhibiting acute peripheral circulatory failure the symptom picture described closely resembles an acute hypo-adrenalism such as is seen in grave cases of diphtheria and the Friedrichson Waterhouse syndrome of meningococcal meningitis?

Lieut.-Colonel J. A. Manifold I have carried out postmortems on cases dying from malignant tertian malaria for some years. My colleagues and myself were struck by the similarity between cases of shock, heat stroke and agid malaria.

We felt that the symptoms could be accounted for by an acute suprarenal insufficiency in conjunction with changes in the brain in the region of the hypothalamus and great nuclei. The brain changes have been beautifully presented tonight by Professor MARGRAITH. The suprarenals in some cases show dilatation of vessels which were comparatively filled with parasitized cells. There was cloudy swelling and some fatty degeneration of the parenchyma, but these changes were not in any way as dramatic as those found in the pituitary. In the latter there was more dilatation of the sinuses, which were more or less completely occluded by parasitized red cells. This is well illustrated in the accompanying microphotographs. It will be seen that there is relatively little change in the cells of the organ itself. This is obviously because the whole process is so acute that there is little time for dramatic and demonstrable tissue changes.

I feel that these changes in conjunction with those in the brain previously referred to are quite sufficient by themselves to account for this "shock like" syndrome in heavy infections with *Plasmodium falciparum*.

Brigadier J. S. K. Boyd I speak with diffidence because my experience of fatal malaria dates back to the bad old days when, as Professor MARGRAITH has observed, our outlook was influenced by the clammy hand of morbid anatomy. Very little stress has been laid on the condition of the heart muscle



Pituitary gland heavily parasitized with *Plasmodium falciparum* ( $\times 300$  approx.)





in fatal cases of malignant malaria. In a series of cases occurring in Salonika in the 1914-18 war, reported by DUDGEON and CLARKE (many of which I autopsied in a forward unit), one of the most striking features was the presence of fatty degeneration in the heart muscle fibres. Forty-five cases are recorded, in 23 of which there was evidence of fatty degeneration—in 5 extensive, in 3 moderate, and in 15 limited. I think there can be no doubt that with such lesions there must be a considerable degree of cardiac inefficiency, capable of giving rise to venous congestion in the liver and other organs. Thus the condition of the heart would be in part, at least, responsible for the production of the anoxia which Professor MAEGRAITH describes. I wonder if Professor MAEGRAITH examined the heart muscle of his fatal cases for damage of this kind.

As regards the adrenals, PAISSEAU and LEMAIRE suggested that many cases of algid malaria occurring in French troops in Salonika were due to adrenal insufficiency. DUDGEON did not agree with this view. He found there was a loss of chromaffine staining material, but nevertheless did not think adrenal deficiency was the cause of algid symptoms.

In other organs small haemorrhages, believed to be related to capillary obstruction by the parasites, were of frequent occurrence. In a number of cases haematuria arising from kidney haemorrhage was a prevailing symptom. Haemorrhage from the bowel was not uncommon. Many cases of malignant malaria had symptoms resembling acute appendicitis, and in the early days a number were operated on with unfortunate results. Ultimately, an instruction was issued enjoining that in no case was appendicectomy to be performed until malaria had been excluded by the examination of a blood smear and confirmatory evidence of an inflammatory condition obtained by a leucocyte count. Small haemorrhages were also found in the lungs and occasionally in the pancreas. I should be interested to hear if Professor MAEGRAITH found lesions of this kind in his cases.

**Dr J W Field.** I should like to ask Professor MAEGRAITH whether he is now prepared to abandon the old assumption that parasite emboli are responsible for focal damage in pernicious malaria. Thirty years ago THOMSON showed that infected cells tend to agglutinate in cultures of *Plasmodium falciparum*. His observation has been amply confirmed since. The phenomenon begins with early schizogony and may progress until there are clusters of infected cells big enough to plug the smaller capillaries and tough enough to resist manipulation in a capillary pipette. A single clump may fill the high-power field of the microscope. There is here a ready explanation for the capillary blockage seen, for example, in some cases of cerebral malaria. Is it not premature to dismiss this hypothesis until it can be shown that the phenomenon does not occur in the body but is restricted to the artificial conditions of the culture tube?

Dr G M Wenyon Professor MACGRAITH has referred to blockage of the brain vessels by infected red cells. This appearance is well known. In the small vessels the entire lumen is blocked but in the larger vessels the infected cells form a layer round the walls while the centre of the vessel is free and contains uninfected cells. It has often occurred to me that much of this blockage may be due to development of malaria parasites which has continued after death of the host so that the appearance of blockage may be a postmortem effect. There is a tendency for the infected red cells to adhere to the endothelial lining of the vessels. I remember that McLAY in Salonika, observed in cultures of the malignant tertian parasite that large mononuclear cells often had numbers of infected red cells adherent to them.

Dr L J Chwatt With reference to Dr WENYON's remark, it may be of interest to mention the results of an unpublished investigation on the time of the apparent survival of malaria parasites in the blood of fatal cases of malaria in African children in Lagos. In blood films taken from the right auricle during the routine postmortems, it was found that *P. falciparum* parasites stain well and are apparently unaltered until 18 hours after death they showed defective staining and signs of degeneration when films were taken 24 hours after death. If the postmortems were carried out 36 hours after death no recognizable parasites were found either in thin or in thick smears.

I should like to thank the two lecturers for their most illuminating papers and to ask Professor MACGRAITH if there is any evidence of specific pathological lesions or their selective localization in fatal cases of malaria caused by parasites other than *P. falciparum*.

It is known that *P. vivax* is not always as benign as its name suggests and there were reports of epidemics with very high mortality rates due to *P. vivax* alone in Russia in 1943. It is also known that *P. vivax* prefers to invade the young red blood cell while *P. falciparum* does not show any preference in this respect. This may suggest that the biochemical requirements of the two parasites are different and that the pathological lesions caused by them need not necessarily be identical.

Dr F Murgatroyd If I understand Professor MACGRAITH correctly he believes that normally the small vessels of the brain, compared, for example with the sinusoids of the liver are highly impermeable to protein, and that small changes in the permeability of the cerebral vessels, following local anoxia, are responsible for the brain lesions of malaria. If this be the correct explanation of the pathogenesis of cerebral malaria one might expect the mechanism to be reflected in an increased protein-content or volume of the cerebrospinal fluid. My impression is that there are not necessarily very consistent or definite changes in the cerebrospinal fluid in cerebral malaria, but perhaps Professor MACGRAITH can throw a more precise light on this matter.

## DISCUSSION

Dr W. D Nicol said he wished to thank Professor MAEGRAITH for his most interesting paper. He was particularly interested in his remarks on those cases which showed signs of cardiac failure which are quite indistinguishable from genuine shock. Cases cited by Professor MAEGRAITH were in association with *P falciparum* infections. In the experience Dr NICOL had had of treating primary cases of malaria in the Malaria Unit at Horton, he had come across some cases with signs of peripheral failure which occurred in cases of *P vivax* infections. Though they are comparatively rare, and in the vivax cases there is a low parasitaemia compared with that found in the falciparum infections, the condition was extremely alarming and gave rise to considerable anxiety. Even after successful exhibition of anti-malaria drugs this condition sometimes persists for several days and he would like to ask Professor MAEGRAITH to give his views on these cases and also on the treatment indicated.

Lieut-Colonel J A Manifold. I feel rather diffident about mentioning this case. A sailor with a heavy *Plasmodium vivax* infection was taken off a sloop in Bombay harbour with a remarkable condition of hyperpyrexia. A lumbar puncture revealed an apparently dry theca. This was repeated without any result.

At the postmortem it appeared that the theca was indeed dry and the choroidal plexus appeared blocked with cells containing large forms of *P vivax*. We are not very certain that this was the true explanation, but the patient had a phenomenal and almost uncontrollable hyperpyrexia.

Professor Maegraith (in reply). The first question was about the part that suprarenal lesions may play in the development of the symptoms of malaria. This is an important point which I did not discuss because of shortage of time. The similarity between the clinical symptoms of certain forms of pernicious malaria and those of adrenal insufficiency have been stressed by many workers, some of whom have considered that the lesions in the suprarenals are primarily responsible for conditions such as algid malaria. PAISSEAU and LEMAIRE (1916) for instance, reported that amongst French troops asthenia and low blood pressure were cardinal signs in certain forms of algid and comatose malaria. On the grounds that suprarenal lesions were always present in such syndromes, these authors considered they were suprarenal in origin. Italian workers, including NATALI (1934), supported this view, particularly in simian malaria. TALIAFERRO and MULLIGAN (1937), in their magnificent work on the pathology of simian malaria, were, however, unable to confirm the frequency of the suprarenal lesions, in many of their cases the suprarenals showed no histological changes. Recently in South America, FLOSI (1945) has investigated the effect of the administration of desoxycorticosterone and sodium chloride in non-fatal attacks of malaria, and has reported that in some cases certain humoral changes, possibly referable to suprarenal dysfunction—such as rise in plasma

potassium changes in the salt/water balance, etc.—were influenced by the administration of suprarenal extracts and salt. The argument that because of the similarity between the clinical symptoms there must be a causal relation between adrenal lesions and the symptoms encountered clinically in pernicious malaria has, however recently been criticised, since it has been shown that medical shock may develop in malaria quite independently of adrenal damage (RICHMOND 1942 HEAN and TAYLOR, 1946).

A few cases of algid malaria certainly show associated severe suprarenal lesions including severe haemorrhage which may appear sufficient to be the cause of death, but this is not a frequent finding. I believe that the humoral changes to which I referred above, do result to some extent from suprarenal dysfunction, which arises from acute reduction of the blood flow through these organs during the malarial attack. The effects of diminution of blood flow are the same as those which have been shown experimentally to follow the exposure of the body to anoxic anoxia.

Lesions in the pituitary have been described by several authors. It is possible that in some cases such lesions may contribute considerably towards the final syndrome but pituitary damage is not invariably present even in the severest cases.

Brigadier BOYD has expressed extraordinary ideas about our approach to this problem. I can assure him that we did examine the hearts in our cases and that a large number of other workers besides ourselves have also done so. The conclusion I have given you tonight regarding the presence of cardiac failure in malaria is not by any means exclusively my own. SPRAGUE (1946), reviewing some thousands of cases of malaria seen in the South Pacific area during the 1939-45 war concluded that malaria seldom caused death by direct myocardial involvement, and that the heart was not greatly affected in patients dying of other complications. HEAN and SMITH (1944) found little evidence of cardiac failure *per se* in 100 autopsies on cases of malignant tertian. SPRAGUE also found no cardiac abnormalities in a series of fifty cases of recurrent *P. vivax* infection. Scattered degenerative changes, sometimes severe are not uncommonly seen in heart muscle in malaria, but one can never say from looking at the cardiac muscle whether it was functioning well in life or not. The only way to decide whether the heart function is affected is to examine the patient. After reading a mass of literature, I have come to the conclusion that cardiac failure is not a common complication of malaria, except as a strictly terminal event. The patient dies more often from vascular than from cardiac failure. The divergence of opinion found amongst authors has led to some extent to confusion between cardiac failure and vascular collapse of the type of medical shock, in which the essential process is quite different from that in heart failure, since the heart, although it can still act as a pump is unable to maintain the circulation because of inadequate venous return. I would like to say that I am well aware of DUDCZOV and CLARK's work. GASKELL and MILLAR at about

the same time and with the same type of patients living under similar conditions, described three modes of death in malignant tertian malaria, in one of which the "pathology centred round the heart". The predominant symptoms were considered by them to result from cardiac failure, but in many of their cases the description given is not that of cardiac failure at all but of generalized vascular collapse.

One of the things which has helped the confusion has been the frequency with which a congestion of liver vessels has been found in malaria. Such congestion of the liver has, in the past, been regarded as a concomitant of right heart failure, but, as MOON points out in his book on shock and capillaries, liver congestion is the rule in shock, in which condition the venous pressure in the vena cava is not raised, but lowered because of the reduction of venous return to the heart. MOON pointed out that such pathological changes occurring in shock have frequently been mistaken by misguided pathologists for the effects of cardiac failure. Brigadier BOYD's mention of small haemorrhages scattered over the tissues is very much to the point. This is, of course, one of the characteristic pathological pictures of acute vascular failure, particularly in the lungs.

I did not say that emboli may not sometimes occur in malaria. Of course they do. SPITZ (1946), has, for instance, reported emboli in some of the vessels of the brain in "cerebral" malignant tertian malaria. Many of these emboli did not completely obstruct the vessel and were found lying flat along the endothelium. The point I tried to make was that the primary lesion in the brain was not thrombosis but reversible physiological stasis.

The matter raised by DR WENYON has been discussed pretty fully. The work of TALIAFERRO and MULLIGAN has shown that these large mononuclear cells filled with parasites and malarial pigment may occasionally obstruct vessels. The accumulation of invaded erythrocytes in contact with mononuclear cells is in keeping with the observations of KNISELY, who has shown that at certain stages of malarial infection these mononuclear cells become "sticky" to the erythrocytes. This may be the result of the general reduction in electrical surface charge which occurs during these infections in both the parasitized and unparasitized cells, or it may have something to do with the deposition of crystals of what KNISELY considers to be fibrin upon the erythrocytes. It is possible, therefore, that both humoral immunity and exposure to anoxic states are involved. It may be, as Dr WENYON suggested, that the state of the parasites seen at autopsy may arise from development of the plasmodia subsequent to the patient's death. This may explain why, when haemorrhage occurs round an obstructed arteriole, the vessel frequently contains parasites which are all at much the same stage of the life cycle, whereas the cells which have escaped from the vessels into the tissues are not heavily parasitized or are free from parasites.

I was very much interested in Dr CHWATT's statement. I think this an important observation. With regard to the evidence concerning the predilection of special species of parasites for certain age-groups of erythrocytes, there is a vast literature. Investigations were started by a provocative remark by EATON (1934) in America—he suggested that vivax merozoites attack only young red cells. This suggestion was made after study of one case only and it bears out my contention that it is at least sometimes worth while to make "synthetic" suggestions of this sort, because, as you know he has since been proved right beyond reasonable dispute. (KITCHEM 1938 and 1939 FERNET *et al* 1946.)

Dr NICOL's observations are important, particularly as he has referred to the appearance of medical shock in vivax malaria. The basic treatment for all forms of medical shock, whether caused by malaria or other disease, is the same. The primary object is to restore the circulating blood volume by the ordinary standard methods, including parenteral administration of fluid in the form of glucose saline or plasma. This is often a life-saver. A fluid intake-output balance should always be kept, particularly in anuric cases, owing to the danger of waterlogging the patient.

In answer to Dr MORGATROD's question, I can say there is not a great deal of satisfactory evidence regarding the protein content of the cerebrospinal fluid in cerebral malaria. The protein of the fluid is usually raised but not very greatly. This is not necessarily an argument against the development of stasis in the brain vessels since a relatively small loss of fluid may give rise to quite disproportionate changes in the circulation.

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The President We shall now close this part of the discussion, but I would like to say once more how much I have appreciated all the hard work that Professor MARGRAITH has put into this paper. Quite obviously he has read everything that is to be read on the subject. His knowledge is very wide. One cannot get away from the fact that, from the clinician's point of

view, the symptoms presented by the patient are due to the concentration of the parasites in one organ. You have heard tonight about the frequency of so-called malarial appendicitis in the Salonika area, and the same thing occurred in Palestine. There the young surgeons then took out their knives and other fellows' appendices, and similar official action had to be taken in that Force. Also I remember a case diagnosed as acute haemorrhagic appendicitis in the Albert Dock Hospital, away back in MANSON's days. It was proved to be malarial embolism of the pancreas. One has seen abdomens opened on the basis of intestinal obstruction in these cases.

We now pass on to discuss Professor SHORTT and Dr GARNHAM's demonstration of the exoerythrocytic parasites of *Plasmodium cynomolgi*.

#### DISCUSSION ON THE DEMONSTRATION

**Major-General Sir Gordon Covell** I have not any more to say than to congratulate Professor SHORTT and Dr GARNHAM most heartily on their outstanding discovery. I was not myself engaged on any of the work carried out on *cynomolgi* in India. This was done by an inquiry under the direction of Colonel MULLIGAN, and I must confess that when I saw the specimens which Professor SHORTT showed me the other day, I wondered how it was possible that they had not been found by the workers in India. The only explanation I can imagine is that the dose of sporozoites which was given out there was not of the colossal size given over here. I do not think the fact that they were looking for something small, whereas the exoerythrocytic forms are in fact very large—I do not think that was the reason. There must be something beyond that, and I can only imagine that the dosage of sporozoites given in India was insufficient for the purpose. I do not know that there is anything further I have to say on the subject, excepting that this discovery makes it all the more certain that the same thing occurs in human malaria, and that it can only be a question of time for that also to be demonstrated.

**The President** It has always puzzled me, if there is a tremendous infection with *Plasmodium vivax*, (that is so closely allied to *P. cynomolgi*), why this cycle has not been already discovered in connection with *P. vivax*. It must occur, and possibly the explanation that Sir GORDON COVELL has given is the correct one, but I would like someone to suggest how we now can verify this in human tertian malaria. It is, of course, not possible to kill the patients and examine their livers. Liver biopsy as practised by members of the Post-Graduate School might give some results, but I doubt whether one would get enough liver by biopsy to enable one to find comparable stages in human malaria.

**Dr F. Hawking** I wish to congratulate Professor SHORTT and Dr GARNHAM on their discovery. Professor SHORTT kindly demonstrated these



parasites to us and then we were able to find them in our own histological material collected from monkeys during recent years. The earliest parasites which we have found are spherical bodies about  $14\mu$  across, situated in parenchymatous cells of the liver. Over seventy pieces of chromatin have been counted in a cross section of one of these forms. The parasites found after 7 days 15 hours, and 7 days 23 hours are much larger the average measurements being  $46 \times 31\mu$ . The liver cell is much distended, being reduced to a mere shell the nucleus however remains large and even slightly hypertrophied. One form was found after 7 days 23 hours which appeared to be liberating merozoites.

Mr P. G. Shute I very much regret that my old chief Colonel S. P. JAMES is not here this evening to see the exoerythrocytic parasites and to take part in this discussion. He was so certain that one day someone would find them in mammalian malaria as he had first discovered them in his chickens some 10 years ago. He so often told me that he believed the liver to be the organ of choice for e.e. bodies.

Colonel SHORTT and Dr GARNHAM's discovery surely means that it is practically certain that these bodies also occur in human malaria and it is probable that in *P. vivax* the bodies will closely resemble those of *P. cynomolgi* because the two species of parasite are almost morphologically indistinguishable. This being so, it would help to clear up the problem of dosage in relation to the incubation period. We know for example, that in *P. vivax* the incubation period is about the same whether a non-immune is bitten by one infected mosquito on a single occasion or by 100 infected mosquitoes once a day for 5 or 6 days. If as I suppose is the case, each sporozoite takes 7 or 8 days to complete its development in a tissue cell then the fever incubation period could not be much less than 6 days. On the other hand, I believe that in *P. falciparum*, quantum of sporozoites does influence the incubation period and we have found rings in the peripheral blood on the 5th day when a million or more sporozoites have been injected intravenously.

Finally we believe that at Horton we have set up a new record by infecting with *P. vivax* malaria parasites a batch of 3,600 *A. maculipennis* var. *atroparvus* and all we are waiting for now is a volunteer.

Professor K. B. Williamson Dr GARNHAM, describing the exoerythrocytic forms of *Plasmodium falciparum*, which he exhibited at the recent Laboratory Meeting of this Society states that a majority of the merozoites liberated from merocytes in the liver of his monkeys "enter red cells to become male and female gametocytes" (*Trans. R. Soc. Trop. Med.* 1943 41 428) and in NOCHT and MAYER's handbook on *Malaria* (1937 page 147) young gametocytes are described as "small, blue, oval bodies in which a ring-like structure, with a pronounced food vacuole is never seen. Since like produces like, there is

good ground for believing that the merozoites from which the young gametocytes arise are themselves compact protoplasmic masses without a pronounced vacuole. But there is no evidence that the precursors of the trophozoites, prevention of whose incursion upon red corpuscles is the prime object of prophylaxis, resemble them. On the contrary, unpublished experiments carried out over 10 years ago in Penang with sporozoites of *P. falciparum* and *P. vivax* bitten into ZAIN and myself go to prove that the trophozoites' immediate precursors are chromatin-headed thread flagellates produced by exflagellation of the sporozoites' chromidia, or by like occurrence in merozoites. NOCHT and MAYER (1937, page 146) point out that the latter may be rod-like, while THOMSON and ROBERTSON (1929, page 17, Fig. 3) depict them diagrammatically as chromatin-containing rings, resembling young trophozoites. It is again a case of like giving rise to like. This raises the question of the true nature of juvenile trophozoites. The accepted view, due to SCHAUDINN (1902, Figs. 47 and 48 of Plate XXII of his Monograph), that they are amoeboid structures, enclosing relatively enormous food vacuoles, is untenable even in the case of closed rings, since food vacuoles are relatively small inclusions within granular endoplasm, and are filled with refractile fluid containing food particles, all of which features are lacking. Trophozoital rings always appear entirely empty except for extruded chromidia and lack both visible roof and floor. The argument applies *a fortiori* to band, comma, and incomplete ring-forms, since being straight lines or open curves they are geometrically incompetent to enclose anything. And the possibility that only the closed rings are vacuolate is precluded, since mature trophozoites, however derived, are structurally similar. What kind of a beast can it be, some of whose young resemble snakes, while others are all stomach and emptiness? Finally, extravagant vacuolation is inconsistent with all that is known of young actively growing cells. What, then, is the true nature of young trophozoites? Combining mutually confirmatory experimental and observational data, it is to be concluded that the young trophozoites are what the eye declares them to be, namely, flagelliform structures, they being sedentary phases of the aforementioned thread flagellates, their hitherto inexplicable diversity of configuration being the result of straight (band forms), or varyingly curved, poses. So a snake may be extended, or partly or completely coiled. And the forked-lightning-like *tenue* forms of *P. falciparum*, which as SINTON showed (*Ind. J. med. Res.*, 1922, 10, 592), appear some hours later than simpler and earlier forms, may be plausibly explained as being produced by free and open branching of the latter. Drawings of thread flagellates observed free, or attracted to, red blood corpuscles in later stages of experimental infection support this general conclusion. One of them shows a double-headed thread undergoing longitudinal division while free in my blood 15 days after infection with *P. vivax*, paralleled by a precisely similar and dividing band form of *P. malariae* (THOMSON and ROBERTSON, Plate I, Fig. 30).

Among other questions calling for discussion, I can only mention one

etc., the possibility that potentially flagellate chromidia present in the blood, may constitute a hitherto unsuspected reservoir of resting and resistant particles which are a source of latent malaria, both incubatory and relapsing. The occurrence of unexplained particulate bodies in malarial blood, first recorded by MARCHIAFAVA and CELLI, lends some countenance to this view.

Thanking you for the forbearance with which you have listened to my obligated testimony and confident of the value of your critical co-operation, I can truthfully say as Sir HUMPHREY DAVY did when conclusions of his were in question, I merely state what I have seen and what I have found.

The President We thank Professor WILLIAMSON for this expression of his original ideas. Everybody has a right to construe things as he sees them.

I will conclude by saying that this has indeed been a historic meeting. We have seen things tonight that few people have ever seen before, and we have heard things tonight that few people have ever heard before. When you can bring into line the physiology of malaria with the parasitology you will have done a great thing and, as soon as the discovery of these tissue forms is made, many of the difficulties we have experienced in assessing the after-effects of malaria may be overcome. We shall have to think hard before telling our patients that they cannot possibly have malaria when they consult us 10 or 15 years after serving in the tropics and insist that they have had it again. We know you can have quartan malaria for as long as 21 years. When people tell me they are suffering from lack of concentration, or that they cannot eat their breakfasts because of having contracted malaria in the recent war I shall perhaps begin to think they are telling the truth.

## COMMUNICATIONS

### BANCROFTIAN FILARIASIS

#### BIOLOGICAL MECHANISMS THAT UNDERLIE ITS PERIODICITY AND OTHER OF ITS CLINICAL MANIFESTATIONS

by

CLAYTON LANE, M.D. (LOND.), Lieut.-Colonel F.M.S. (retd.)

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# BANCROFTIAN FILARIASIS

## BIOLOGICAL MECHANISMS THAT UNDERLIE ITS PERIODICITY AND OTHER OF ITS CLINICAL MANIFESTATIONS

BY

CLAYTON LANE, M D (LOND), Lieut -Colonel I M S (ret'd)

### INTRODUCTORY

On theories regarding the causes of microfilarial periodicity, POYNTON and HODGKIN (1938) wrote that the diurnal periodicity of *Loa loa*, the general nocturnal periodicity of *Wuchereria bancrofti*, coupled with the non-periodicity of the Fyian form whose microfilaria is "apparently identical with *Mf bancrofti*, seem effectively to dispose of any theories that have so far been put forward, and that moreover the matter is of little importance except as a fact."

But when, after MANSON had discovered this condition, "COBBOLD made it known at a meeting of a well known scientific club in London, it was received with bushels of chaff, a Gratiano present displaying one of his few grains of wit in the question 'whether the filariae carried watches'" (MANSON-BAHR and ALCOCK, 1927, page 40) Something, so unexpected that its first report was ridiculed, is now dismissed as of little importance. On the contrary, its right interpretation is essential for that understanding of filarial infection which it is the duty of Tropical Medicine to have, and MANSON himself held that "filarial periodicity ought to be full of significance to the general practitioner"

It is also hoped, in what follows, to convince that these differences in filarial periodicity do not dispose of every theory regarding its causation that had been put forward before 1938

### SECTION I

#### THEORIES AND THEIR CONTROL

"To use the past in order to interpret the present      this is the way of wisdom"  
—INGE, 1933

Theories on this matter have been insufficiently based on histological study, and the quality and variety of some 800 filarial sections that have come to me by the late Professor F W O'CONNOR's gift and Will give the opportunity to do so, and to place at others' disposal facts and inferences based on their



examination, hoping that more observation and less speculation will underlie future writing on microfilarial periodicity. I suggest that the present attitude on this matter is too like that of Greek philosophy in the fifth century A.D. when "thought was still largely under the dominion of imagination not leading it and controlling it, but starting from it and controlled by it" (TEMPLE, 1929, page 323). Because imagination has been so free in the explanation of microfilarial periodicity and scientific control so little considered, it is here felt necessary to try to make clear how much needs to be set aside before the mechanisms of periodicity can be understood and hope of combating their ill-effects set on a reasonable basis. The mechanisms of parasite and host that underlie microfilarial periodicity are then considered.

### 1. MYERS THEORY (1881)

MYERS held (1881, 1886) that the perturbation of female worms was continuous, that the young remained in the lymphatic system for 12 to 24 hours, and then, urged by favourable conditions in the circulating blood, exhibited for the first time "selective ability" entered this, and caused the rise of the microfilarial blood tide. The brood might come from many mothers and be too great for thousands of mosquitoes to get rid of even if they were constantly at work, so he suggested that such microfilariae as escaped mosquitoes were dissolved when day came.

#### CONTROL

For quiescence of the microfilariae in the lymphatics by day and for what, had he lived today, he might well have called "nightly toxæ" to blood, MYERS offered no control for their solution by day as the cause of the tide's fall. He offered as control the percentage of microfilariae that survived solution for 120 hours after blood had been drawn. In blood drawn at 01.30 hours the percentage of survivors was 64.5 (20 of 31). In that drawn at 07.45 to 08.00 hours it was 0 (0 of 19). Of these experiences STARRIEB & MACDONALD (1887) got confirmation, but BLANCK (1887b) did not.

#### DISCUSSION

Evidence is offered below for the conclusion that microfilariae are born simultaneously in numbers just before the blood tide begins to rise at night, and that its fall is due to their destruction by the activity of at least the visceral part of the reticulo-endothelial system. To this, having reached the blood, they would be carried again and again. It is reasonable to conclude that the more numerous are the microfilarial entries into the destructor organs the more will they be damaged and the shorter will be the period that they will remain alive and undissolved in drawn blood that contains them. Microfilariae in blood drawn soon after midnight have passed through the most dangerous organs and tissues far less often than those which have survived in the circulating blood till morning, and with this MYERS experience agrees.

## 2 MANSON'S THEORY (1882)

MANSON (1882a) set out the problem in these clear words

"The facts connected with filarial periodicity may be best explained by one of two suppositions (a) the parent worm empties her uterus of mature embryos once in 24 hours, parturition going on from late in the afternoon till midnight and, as a corollary, the young filariae living but a few hours in the blood, as Dr MYERS suggests, or (b) parturition is a more or less continuous process, the young being nearly constantly carried along the lymphatics and thoracic duct into the blood. In this they live for an indefinite time, circulating with it, under ordinary circumstances, during the night, but, from some unknown cause, becoming fixed during the day. The hypothesis of intermittent reproduction implies but a very short life to the embryos, but it is difficult to understand what purpose could be served by this enormous daily mortality. Nature's object in making these creatures so prolific is evidently to provide as many chances as possible for the continuance of the species. Nor is it easy to understand why animalcules which can live for so many days outside the body of their host should die after so short a life in it. The facts I have stated, and other evidence I do not bring forward at present, lead me to believe that the second hypothesis is the correct one—*viz*, that reproduction is continuous, and that the embryos are fixed and filtered out in some organ or tissue during the day."

## CONTROL

Sixteen years later the result of a necropsy confirmed MANSON in his conclusion "A recent opportunity has enabled me to ascertain that, during the diurnal temporary absence from the cutaneous circulation, filariae\* retire principally to the larger arteries and to the lungs where during the day they are found in enormous numbers" † (MANSON, 1898)

## DISCUSSION

MANSON held that microfilariae are being born continually, but that all present in the blood at morning time retire for the day to the larger systemic arteries and lungs, and so cause the fall in the microfilarial blood tide, with the coming of night they re-enter the general circulation and, with those then being born, cause that tide to rise.

In his great pioneer work MANSON had few known facts to guide him, as is evident from the derision that met the announcement of the most striking of them all—microfilarial periodicity, but he set out with characteristic lucidity the two possible causes of this and the reasons for his choice, yet, as noted below, he became the victim of circumstance by the very findings which in the knowledge of 1898 seemed to furnish the control he sought. Increased knowledge forces reconsideration in three directions of the basis of his conclusions

## MANSON'S ACCEPTANCE OF CONTINUOUS PARTURITION CONSIDERED

MANSON'S conclusion that parturition by *W. bancrofti* is continuous seems first to have been published when dealing with Case LXI (1882b = Case IV, 1883), whose microfilarial numbers he recorded every 3 hours for 144 hours, those in the blood by counts of smears measuring 1½ by 1 inches, and those

\* In present usage microfilariae

† Average microfilariae per slide were lungs, 675, carotid artery, 612

from chyle in a drop of the deposit in chylous urine. He added together and tabulated (MANSON 1883 page 69) the numbers present at the same hours in blood, and in lymph as measured by deposited chyle in urine.

In blood there was the normal periodicity with its peak at night. Of the lymph figures he wrote: "It seems to me that they indicate that filarial embryos are nearly constantly passing into the lymph stream."

Therefore filarial periodicity is independent of the act of parturition which is more or less continuous process. But when these figures are set out as Graph (Chart I) the numbers of microfilariae in the lymph, though some were always there were not at a nearly constant level but have day-time peak, and night-time fall nearly but not quite to zero. Moreover in his Case XIX (1883) the microfilarial lymph curve did fall to zero here MANSON had aspirated varicose lymph nodes in the left groin at 08.30 hours and got abundant active microfilariae while such aspiration at 19.00 hours gave him none from either side. Here again the blood periodicity was normal while the lymph periodicity had morning peak and complete night fall. These figures established the microfilarial content of lymph which, so MANSON put it, was on its way to the blood by retrograde compensatory track. Lastly regarding Case XXII (1883), MANSON drew off fluid from lymph scrotum at 11.00 hours, at 16.00 to 17.00 hours, and at 19.00 hours and found two or three microfilariae on every slide (there were none in the blood) and wrote that they observe "no periodicity while they are in the lymph, and that reproduction is continuous process." His biographical summary runs thus: "Examinations of the dog had shown that the embryos of *Filaria* [*Dirofilaria*] *immitis* did not die off during the day time, and continuous examination of lymph from an oozing lymph scrotum and of chylous urine showed by persistence of embryos in these fluids during the daytime that the process of parturition in *F. bancrofti* once begun was not suspended in the daytime the periodicity therefore must depend on circumstances existent in the blood (MANSON BULL and ALCOCK, 1927 page 60).

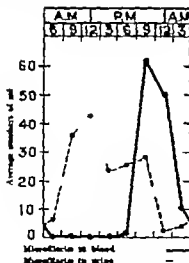


CHART I. Average counts of microfilariae in blood and in chylous urine made at the same hours on six consecutive days. Drawn from the numbers in MANSON's paper (1883b)

From the writer's paper in the *Lancet*, of 21/4/27

To sum up, in MANSON's Cases IV and XIX periodicity of microfilariae in lymph was emphatically present; it differed from that in the blood by having a morning peak, and in the latter case the periodicity was complete in some lymphatics as was that of the blood periodicity of *Lee* loc. The significance which MANSON himself set on such timing in the blood is shown by his renaming of this parasite as *Lee disease* and by his giving



even context with persistent presence of the young. None of this is evidence for periodic parturition, but none of it excludes this. It is not acceptable evidence for continuous parturition.

#### MASON'S REJECTION OF PERIODIC PARTURITION CONSIDERED

TINA has met both MASON's difficulties on this matter. The reconciliation of long microfilarial life outside host's body with short one in it lies in ASCHOFF's conception, in 1924 of the reticulo-endothelial system as an entity. Its action in the mechanism of periodicity is more closely considered below; it need merely be said here that its active cells destroy microfilariae provided they are sufficiently near to them. In the ample spaces of hydrocele or of dilated lymph vessel this need not be so for in these the active cells, at least in sufficient number do not pass the wall. Their prey is caged and the lions are shut out. This consideration equally meets the difficulty felt by SUMNAR RAO (1937) when having over 24 hours made 4-hourly examinations of hydrocele fluid he found the numbers of microfilariae per c.c.m. nearly constant, and added, "This seems to indicate that in closed sac like the tunica vaginalis there is no periodicity as is assumed in the theory of cyclical parturition." But periodicity of microfilarial births does not presuppose periodicity of microfilarial numbers in hydrocele fluid. A periodicity of births into any sector of the lymph tract will not be manifest downstream from their birthplace as periodicity unless the stream between these two spots is through, is free and is even. Fluid that seeps into the normal tunical cavity escapes along the lymphatics on the spermatic cord. If there should be obstruction at some spot to the free flow through them, the fluid will collect upstream of that spot as hydrocele and if female worms are giving birth to young directly into the hydrocele it will show periodic increase in microfilarial numbers in the collected fluid. But if microfilariae are there because the lymph current has not only been dammed but has even been reversed, conditions are not merely abnormal, but the extent of their abnormality is necessarily unknown in adequate detail, and inference from the abnormal to the normal is inadmissible.

The inference of SUMNAR RAO that the microfilarial content of hydrocele fluid would necessarily show periodicity if the rise of the microfilarial blood tide were due to periodic maternal parturitions, I cannot accept.

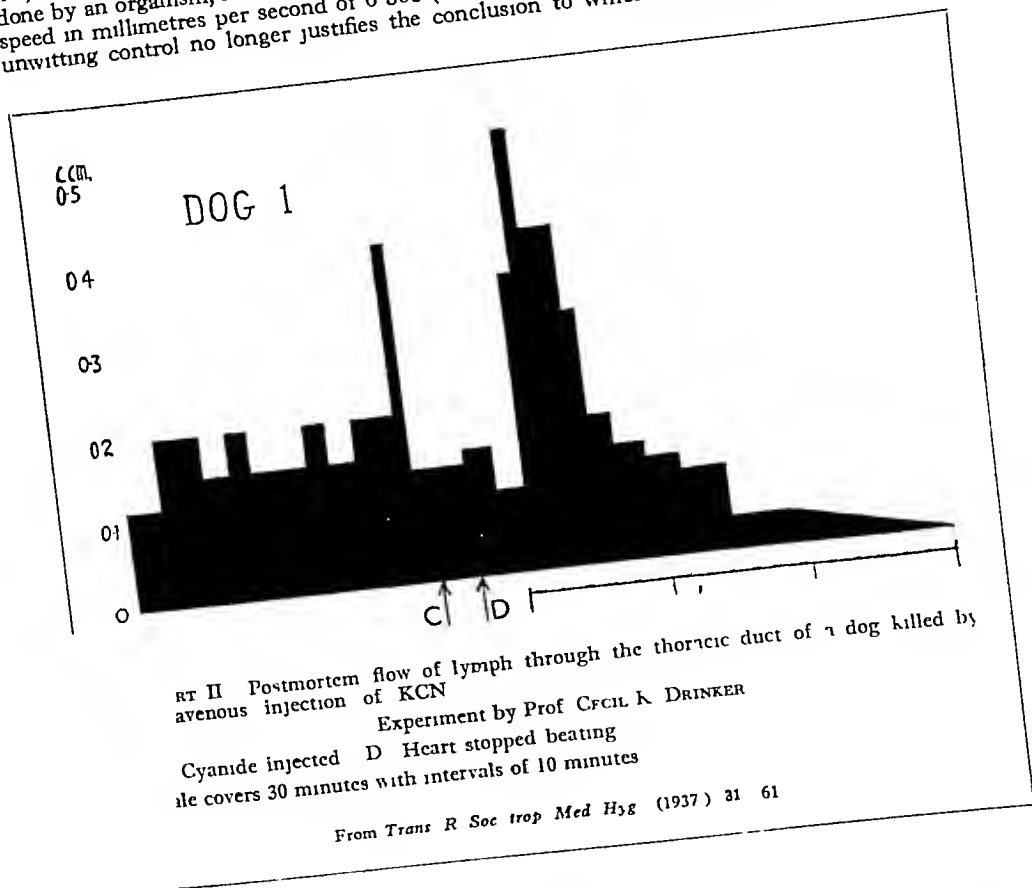
MASON's belief in the purposelessness of heavy daily mortality of microfilariae looks at the matter from the viewpoint of the parasite welfare that if the host calls for larval destruction. The activity of his reticulo-endothelial cells against different animal parasites in his tissues has increasingly been reported during the last few years, and it holds abundantly for *B. bancrofti* before and after it gets into the blood. The species has survived just because it is sufficiently prolific for there to be in the small quantity of blood that mosquito extracts for meal enough microfilariae to infect, but not enough to kill it before they have reached the stage of infectivity for the next victim. If the risks to an existing species during its transfer from host to host are great, it will have survived only because it has become sufficiently prolific to surmount them: *B. bancrofti* has survived.

#### MASON'S CONTROL CONSIDERED

MASON's conclusion that he had discovered the daytime haunt of microfilariae, which was required by his hypothesis, depended on the assumption that at the time of legal death the flow of lymph ceases—that the state of affairs found in the lungs and great systemic arteries 6 hours after death—in this case after fatal cyanide poisoning—was necessarily that which had been present at the time of his death. That assumption was at that time justifiable and on it rested MASON's belief that he now had proof of the rightness of his theory. The advances in knowledge that have rendered that assumption no longer justifiable may briefly be re-stated.

Eight years later BARONOW (1906) found that lymph might flow after legal death, and in answer to question of mine as to lymph flow after death from cyanide DIXON put through and courteously placed at my disposal (LACE, 1935, 1937) details of experiments which showed that in dogs killed with potassium cyanide the lymph flow far from stopping on death, was markedly increased and was prolonged after it (Chart II). The

demonstration allows of two possible explanations. Either experiments on dog are not evidential for man either here or in the deliberate production of elephantiasis below noted—a delight for “antivivisectionists” but inconsistent with the advances in medical knowledge that dog has in such ways given to man—or a great postmortem flow of lymph must be expected to have taken place in the man who unknowingly made himself MANSON’s control. Only the latter seems to me worth serious consideration. Granting this, a similar flow of lymph after this man’s death would have carried microfilariae—present in the thoracic duct when the man died, or entering it afterwards while the flow continued—to those very places where MANSON found them. If they were there during this man’s life, there must be some unknown and mysterious physical means by which this can be done by an organism, muzzled by a sheath, possessing no holdfast, and having a measured speed in millimetres per second of 0.363 (0.243 to 0.479, O’CONNOR, 1936). MANSON’s unwitting control no longer justifies the conclusion to which it seemed to lead in 1881



## SUMMARY

Reasons have been given for certain conclusions. Firstly, in accepting nutrition as continuous, MANSON can hardly have escaped being influenced. It is believed that in the usual infection only one female worm was present, that deductions as to local lymph currents in obstructed lymphatics could

safely be made during a host's life. Secondly at a time when ASCHOFF's conception of the reticulo-endothelial system as a destructive and defensive whole had not yet been formulated, MANSON could not know that a long life of microfilariae outside the body was not evidence that an equally long one was normal in it nor did he realize that though a great microfilarial mortality served no good purpose for the parasitic species, it served the host well. Thirdly he could not know that what seemed to be a fortunate control was vitiated by the ignorance of every one at that time that lymph flow did not cease on death but that, on the contrary in the peculiar circumstances in which that control was unwittingly put into action when the man took his own life, postmortem flow of lymph was prolonged and even increased, so that after the man's death it would have carried microfilariae in it to the unexpected positions in which MANSON found them. His findings are no longer acceptable evidence that microfilariae harbour by day in the lungs and great systemic arteries, and so produce periodicity by their disappearance from the skin blood.

### 3. LANE'S THEORY (1929).

MANSON had set out two reasonable alternative explanations of microfilarial periodicity had chosen that based on a continuous birth of the young turned into periodicity by the fixing of the larvae somehow and somewhere in the host's body by day and had identified that site as the lungs and great systemic arteries. His views appear to have been unquestioned for nearly 50 years. That calm was broken by a paper (LANE, 1929) which, reasoning thus, chose the explanation which MANSON had rejected. If microfilariae are absent from skin blood by day because they have withdrawn to some other habitat, it is physically unthinkable that this should be the overwhelming current of the great systemic arteries, for the larvae have no mechanism by which they could attach themselves to the vascular walls. It must have been after the death of MANSON's patient that they got where MANSON found them.

As to the mechanism of a possible daytime withdrawal from cutaneous to deep capillaries, their coming to a stop in the latter must either be by periodic contraction of these, if they can contract, or by periodic posturing in them by larvae during the day. These forces would have to come into action by day only when infection was by *W. bancrofti* in most parts of the world, by night only when it was by *Loa loa*, and at no time when it was due to *Acanthocheilium pernix* or to *W. bancrofti* in the Fiji region. What would happen to the selective vascular mechanism in a double infection was beyond LANE's imagination again if the effect were produced by larval posturing at certain hours. This was at once discarded when the blood was drawn and lastly the use of no such daytime haunt by *W. bancrofti* had been demonstrated during 50 years of study. After all, of what use would such daytime

The mechanism effecting this was established 6 years later by DANCRA.

## CLAYTON LANE

withdrawal from the skin be to the species. If carried from man to man by a night-biting insect the larva must be in the skin blood at night, but how does it profit it to be out of this by day?

So LANE turned to and, by exclusion, accepted the other hypothesis (considered possible but rejected by MANSON), namely, that periodicity must be due to simultaneously timed parturitions once in 24 hours by the mother worms, the young being destroyed each day. Moreover, this periodicity was more than an adaptation to the habits of a night-feeding insect. It was something that produced in the blood at the night hours a concentration of microfilariae sufficient to infect the rather refractory insect host present in those preponderating parts of the world where the infection is periodic, but insufficient to kill it by hyperinfection. LANE, that is accepted MANSON's view that periodicity was best explained by one of two hypotheses and having, he was satisfied, excluded the one MANSON chose, accepted the other which MANSON had rejected.

His questioning of tradition had two effects. The first was the appearance of the other theories discussed below, the second and more important was the rejection of the late Prof F W O'CONNOR to my appeal for further investigation, before his untimely death he had collected a mass of material got at operation and necropsy, had published little on its examination, but had, in addition to gifts during his life, left me by Will the great number of slides already mentioned.

## CONTROL

The study of these slides forms the foundation of later sections of this paper and the control of my theory.

## POYNTON AND HODGKINS THEORY (1938)

For the present purpose consideration is needed of these writers' views on the causation of blood tides and of inflammatory attacks of this infection.

## (i) THE MICROFILARIA BLOOD TIDES

They write: 'It is convenient to regard the periodic parturitions of the larvae from the deep vessels and lungs to the surface as a characteristic acquired by insects on that is linked with the biting habits of the vector mosquito.'

## (ii) THE INFLAMMATORY ATTACKS - THEIR RHYTHM AND CAUSE

(a) *Theory*

The writers studied this rhythm in detail. They give good examples and state that these periodic attacks occur at regular intervals, every 48 hours, and that even on the 12th less than one attack occurs in 48 hours. They also state that such a high percentage of cases are noticed that these attacks occur at regular intervals that they are periodic.



at regular intervals, and as a result of successive migrations through the walls of the lymphatics to the blood stream [vessels of] the latter become thickened and impassable with the result that the microfilariae die *in situ* and set up an intense local reaction.

Comment.—None was reported.

### DISCUSSION

However acquired, the microfilarial blood tide is looked on by the writers as unquestionably due to migrations of microfilariae between deep and skin vessels. It has been noted above (page 726) that our imperfect knowledge of today is yet so far ahead of that available to MAXSON when he drew these conclusions, as to forbid any correction that the tides may be explained by microfilarial migrations.

Regarding patients ideas of regular rhythm of inflammatory attacks, it is over 60 years since MACROFT (1833-83) wrote thus of the most regular of them. Some writers maintain that the recurrence of the attacks of lephantoïd fever bears certain relation to the lunar cycle. I have often inquired about this from my patients, but never succeeded in establishing any proof of such relationship. But their wide examinations have left PORTYER and HODGKIN satisfied that in their patients these attacks had regular time rhythm, not the same in all hosts, but much the same over series of attacks in the same host. A possible explanation of that rhythm is considered later.

As to their suggested cause of inflammatory attacks, BARR (1912) had already written in his Appendix XIX

Periodic discharge of microfilariae may be a factor in the production of lymphangitis, orchitis and furunculitis. The facts of the case related above would suggest that the sudden outpouring of microfilariae into the glandular substance [of lymph node]

and into the tunica vaginalis was connected with the respective attacks of orchitis and orchitis. Again O'CONNOR (1931) wrote of his Case V. Every phase of transit through the wall of the lymphatic could be seen. The sheath could be made out in some sections and in a few it seemed to be enlarged or ballooned anteriorly.

The mechanism by which the microfilariae migrate from the vicinity of the parent worm in the lumen of the lymphatic through the walls of the same lymphatic to the neighbouring small blood vessels on their way to the general circulation has been demonstrated. While it is not claimed that this is the usual method of migration, the determined nature of its performance suggests that this might be the case.

For over 30 years there has, then, underlain the views of certain workers the conception that microfilariae are suddenly outpoured into the lymphatics, and thence pass thereon into the lumen of blood vessels, and to this PORTYER and HODGKIN added the suggestion that it is the death of microfilariae during this migration which causes the attacks of acute inflammation. O'CONNOR believed that in sections of lympho-varicocles from V. he had visual evidence of microfilariae in process of this transit from lymph to blood vessels. Critical consideration (of what is an inference) is wrapped up in the fate of the microfilarial sheath during tissue penetration. Thus larval sheath (just as is that of hookworms, of strongyloides and of other nematode larvae) is separated but still enclosing cuticle cast at the last ecdysis.

Before further development the larva escapes from this, and it does so by passing with its head the end of the sheath against some obstacle and by boring through it towards or into that obstacle. For Jiff *beaucoup* this is normally the wall of the mosquito midgut, the larva continuing development in this insect body after leaving the sheath behind in the canal. (turns to be passed later in the droppings). That this thigmotaxis urge persists even in the definitive host is revealed by the presence of cast sheaths in that host viscera (Figs. 1 and 2). Of the commonness of unheathing in the definitive host no estimate is possible. There must first come into wide use an adjective for the chitoid of which the sheath is made, for even such considerable lengths of shed sheath as these are hard to pick up and it is likely that short lengths or transverse sections of others, perhaps of many others has been missed.

Since the matter of tissue penetration, without loss of their sheath by nematode

1934), nor was it recovered from the blood unless septicaemia had set in, and then it was present in all parts of the body (DRINKER, FIELD, WARD and LYONS, 1935). In dogs these attacks are bacterial but this can be determined only if there is local examination at their very beginning, in British Guiana the septicaemia was well known under the name "abdominal filariasis" (ANDERSON, *et al.*, 1924). In the discussion on the paper by HOMANS, DRINKER and FIELD (1934), W. A. OCHSNER reported from New Orleans that streptococci were isolated from several persons during such attacks and that a polyvalent serum, given during an attack and thereafter monthly for a year, had usually prevented their recurrence and stopped progress of the elephantiasis. SUNDAR RAO, too (1937) has reported recovery of streptococci during acute attacks, with cessation of recurrence of these after getting rid of septic foci and giving anti-streptococcal treatment. Apposite is the experience of SAMPSON HANDLEY (1909) who, nearly 40 years ago, led silk threads from ankle to iliacus muscle in an elephantoid leg, with lessening of its circumference at the ankle from 21 to 14½ inches, but suppuration set in on the 9th day, and 3½ months later, when the patient's health was good, diplococci were cultured from the blood of the arm and from the lymph of the elephantoid leg. They disappeared under vaccine treatment and a second operation was undertaken, again to be followed after the same interval by a slight rise in temperature, but vaccine and lowering of the leg ended the trouble, and it was concluded that the infective organism had been endogenous. Apposite, too, is a report by KNOTT (1944) on twenty-two patients in the Cook Islands, with abscesses of thigh, buttock, abdominal wall and axilla, often with large metastases. In these patients had microfilariae in the blood or showed clinical signs of filariasis. The conclusion to which the available evidence points is that it is to be added bacterial infection that are due the acute lymphangitis and acute lymphadenitis so apt to occur in this infection. Does a short immunity to this bacterial infection become established?

#### SUMMARY

The theory of POYNTON and HODGKIN adopts MANSON's conclusion that the microfilarial blood tides of this infection are due to periodic larval migrations between deep and surface blood vessels. The error in MANSON's supposed demonstration of this migration has been discussed above (page 723). The writers further postulate that the inflammatory attacks (common features of this infection) are due to the following sequence of events—periodic massive births of microfilariae, their prompt penetrative and massive migrations through tissues from lymph vessels to blood vessels, in this journey the migrants suffer massive death which sets up inflammation. For this sequence they admit lack of proof, for it they offer no evidence, but the evidence of other observers links these inflammatory attacks with superadded bacterial infections.

Finally, for the species to survive as it has done and still does, microfilarial numbers in the blood must be kept up by new births in sufficient numbers to make good wastage. If these births take place periodically at the time of, and are the cause of, inflammation and fever, it is unreasonable to expect that none will reach the blood to make good that wastage, and so will cause at these times an increase in the microfilarial numbers that are counted circulating in it. There is, so far as I know, no report of such increase. On the contrary, time has confirmed MANSON's original observation that with these attacks there may be expected a fall in these microfilarial numbers.

I have, I suggest, rendered this unsupported theory one that is contrary to observation and inference.

## THE MICROSCOPIC APPEARANCES OF FREE YOUNG ALREADY DEAD.

The main changes in free microfilaria dead before fixing are that the body becomes wider its cuticular striation more obvious, its general outline irregular its nuclei are dispersed, enlarged and perhaps faintly ring-shaped with more deeply stained spot—signet ring shaped in fact further dissolution leaves mere microfilarial outline with spots in it. These appearances (Figs. 8 and 50 to 54) have been brushed aside as artefacts there is here then, it is suggested, artefaction in many parasites, but curiously enough artefaction limited to parasites, the nearby host cells looking completely healthy. In Sal. many microfilariae were calcifying (Fig. 9) showing deep diffuse haematoxylin staining and fracturing, affecting perhaps part only of microfilarial length, and in one larva producing, round one fracture an appearance as of a callus (Fig. 10). Flat-coiled embryos show these same changes, but calcification has not been seen. Unembryonated eggs found in 0 to 100 per cent. of sections from different persons (see Table) are larger than killed intra-uterine eggs, their nuclei become ill-defined and disappear no calcification of them has been seen.

TABLE.

OCCURRENCE FREE IN TYPIC NODES OF YOUNG STAGES OF *W. bancrofti*.

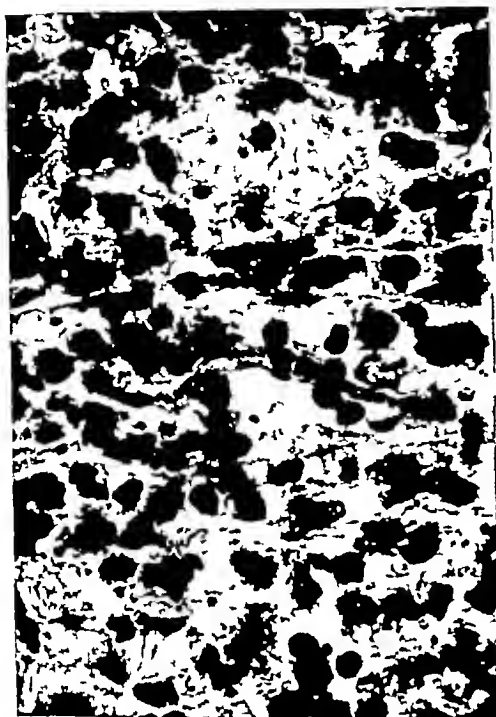
Name	Number of sections containing young	Percentage of sections with		
		Microfilariae	Flat-coiled embryos	Eggs
Mal.	85	100	33.8	37.1
Cog.	42	93.8	79.8	83.3
Hol.	26	100	100	7.7
Sal.	69	100	13.8	9
Re.	4	75.0	37.5	25.0

O Common sectioned lymph nodes from seventeen infected persons. Only in the five here tabulated are free filarial young found, and the node sites were Mal., sub-lingual, inguinal, and those of both sides; Cog. right inguinal; Hol., periaortic and above diaphragm; Sal., right inguinal; Re., right and left sublingual.

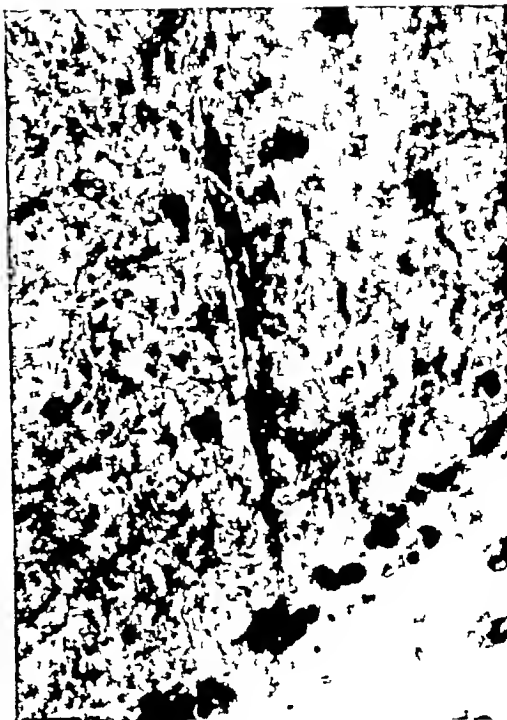
For the suggestion that the acute inflammatory attacks now under consideration are due to massive birth of microfilariae, to their subsequent burrowing through tissues and to their death therein, no evidence has been found, and there are strong reasons against such an explanation. Acute inflammation with fever has commonly a bacterial origin. Has it so here?

## EVIDENCE FOR A BACTERIAL CAUSE OF THEIR INFLAMMATORY ATTACKS.

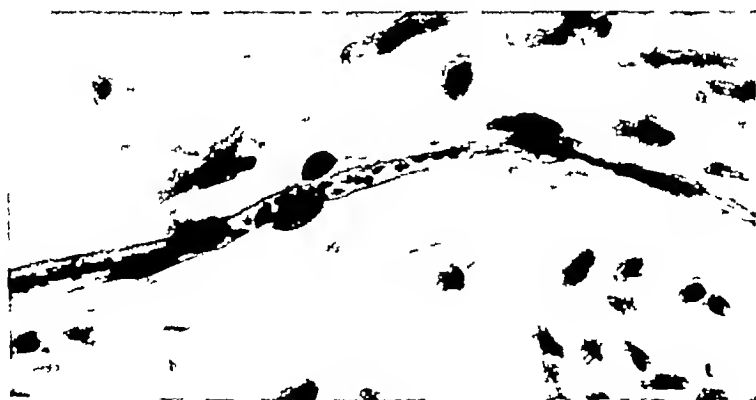
McLINTY (1931) thus summarized his study of thirty-eight cases of the acute inflammatory attacks that occur (mainly at least, in lephantoïd limbs) in this filariasis. "We believe that the almost uniform absence of positive cultures in this series of cases of acute filarial lymphangitis studied under such aseptic technique indicates that bacterial invasion does not always or even generally account for the acute manifestations in this condition. Next in type come reports from DRINKER's laboratory that, when elephantiasis had been produced in dogs by occluding lymphatic injections, there might occur as in man, acute inflammatory attacks in the elephantoid limb and that haemolytic streptococcus was isolated from the oedema fluid within the first few hours of an attack but never at any other time (DRINKER, FIELD and HOMANS, 1934; HOMANS, DRINKER and FIELD



1



2



3

FIG. 1.—Microfilarial sheath cast in the host's spleen. From Mul.

FIG. 2.—The tail of a microfilarial sheath. Similar to Figure 1, showing the dense, granular texture of the tail.

## 5 KHALIL'S THEORY (1935)

KHALIL's theory of the cause of microfilarial periodicity is found in a paper with long corrigendum (KHALIL, 1933a) and with further corrections in a later paper (1935b). It must assume more or less continuous parturition, for to cause periodicity it calls in the special features of sitting of mother worms, of gravity and of a periodic flow of chyle. With mother worms in the upper limbs, so it is held, the lymph flow will carry their microfilariae quickly and continuously into the great veins. With worms in the lower limbs it will take them into the receptaculum chyli, and while the host is upright they will stagnate there when he lies down at night gravity will cause to precipitate them, and their passage onwards will be helped by the after-food flush of chyle which was at first stated to be greatest 1 or 13 hours after food (this timing requiring that the main meal should be a midday one). However KHALIL (1935b) acknowledged misreading his authority which said that the after-food flow remains greatest for the 12 or 13 hours that follow meal. The interaction of the two forces of gravity and chyle-flow KHALIL believed to carry the bulk of the microfilariae into the blood about midnight.

CONTRACT. None was published.

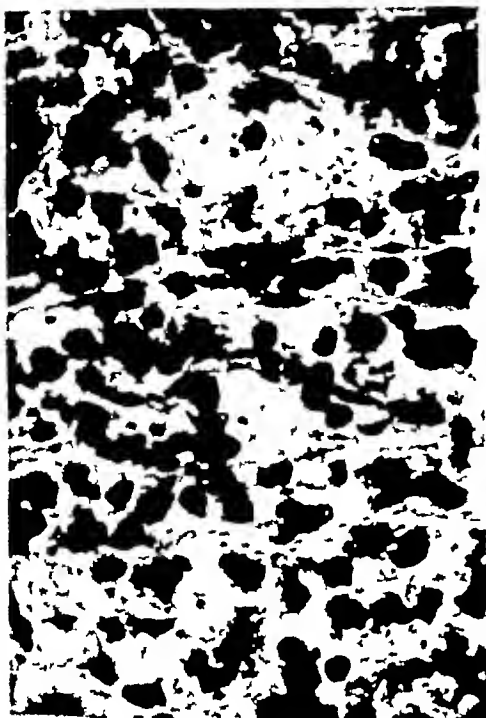
## DISCUSSION

(1) The supposed sitting of adult worms is based on a statement attributed to MASON-BARR who so KHALIL puts it, found elephantiasis in 33 to 75 per cent. of persons with the non-periodic filariasis of Fiji. The text in question reported on thirty males and eight females and it says that of the latter "in or about three-quarters had elephantiasis of the arm as against a third among the male cases" (BARR, 1912, page 40). These insignificant numbers actually lie in a section with the significant heading, "The lower limbs and scrotum are the parts of the body most frequently affected" with elephantiasis in Fiji. Moreover in his Appendix 11 BARR analysed in four groups various clinical signs of filariasis detected in Fiji among 1,333 persons (817 males and 516 females) and found inguinal nodes enlarged in 52.5 to 60.2 per cent. Further he examined 1,624 persons for enlarged epitrochlear nodes and found this condition in 22.2 per cent. only. If the one physical sign is as good an index of worm sitting as the other the larger figures will give the true picture.

BANCROFT's work (1928) afforded further evidence that sitting of other accepted clinical signs of filariasis has no bearing on periodicity. Longitude 170° E. roughly divides the periodic filariasis of the New Hebrides to the west, from the non-periodic form of Barron and the tofts to the east, over the whole area syphilis is absent and yaws in childhood is universal, but the lymph node enlargements which the latter causes has subsided by the time children have grown up, so that these enlargements become there a specially valuable index of the sitting of parent worms in adult hosts. In the non-periodic area BANCROFT found epitrochlear nodes enlarged in 49.7 per cent. of 1,056 persons, as against 23.9 per cent. examined in the periodic area—apparent evidence in favour of KHALIL's theory the difference being 2.1 to 1. But in different parts of the non-periodic area itself, there are equally great differences, the epitrochlear nodes being enlarged in 63.2 per cent. of 653 Samoans and in 29.1 per cent. of 413 inhabitants of the atolls, or 2.1 to 1. Seeing that there is the same percentage difference between different parts of the non-periodic area as there is between that area as a whole and the periodic one, sitting of adult worms as so judged cannot be significant in explaining the presence or absence of periodicity. The variations must have some different cause.

(2) As to the influence on periodicity of an increased chyle flow resulting from the hour of the main meal, KHALIL misstated his authority when he put forward his theory and he made no control. MASON 55 years earlier had taken up the point and, as was his wont, had controlled it. Filarial periodicity is maintained when the hours of eating are changed so that the middle [7 main] meal is taken at midnight and not, as usual, at midday (MASON, 1883, page 59).

(3) Posture as a factor in producing or not producing periodicity would readily be controlled by medical men who have put to bed, for some relatively trivial complaint, patient showing filarial periodicity. Such unconscious controls must have been frequent in the past. There is no evidence that they have shown any effect on periodicity.



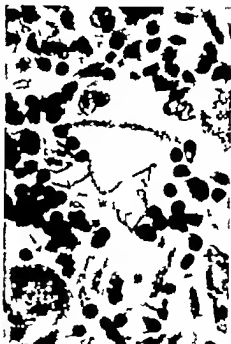
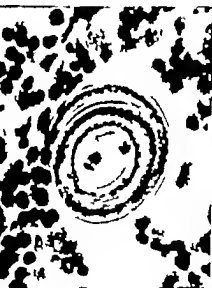
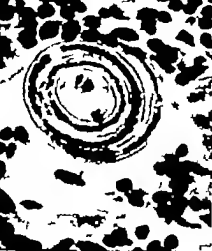
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- 4.—An almost complete killed, flat-coiled embryo seen on the flat in left subcapsular lymph node. Its head end is peripheral; the whole appearance roughly suggests target. From Mul.
- 5.—Cross-section of free flat-coiled embryo in mass of low pre-aortic lymph node. The egg membrane is stretched across the coil. From Hol.
- 6.—The target-shaped embryo seen in Fig. 4 is so focused as to show that with the outstretching of the larva the egg membrane has begun to tear at the "bull" eye.
- 7.—A structure judged to be an empty and partly collapsed egg membrane after escape of embryo; it lies among collection of young forms—flat-coiled and outstretched embryos and degenerate eggs in groin node of Mul.



8



9



10



11

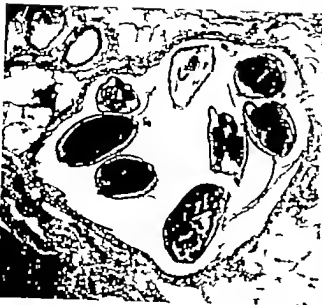
FIG 8.—Early effect of microfilarial dissolution the larvae having lost their normal sharp definition. The host's cellular reaction includes the local appearance of giant cells a change rare in lymph nodes in O'CONNOR'S material. From the marginal sinus of a lymph node from Mul.

FIG 9.—Degenerate and calcified microfilariae in granulation tissue forming a false papilla at the drainage angle of a tunica vaginalis. From Sal. The calcified young show fractures.

FIG 10.—Rounded fracture callus like deposit has formed and the microfilarial nuclei show faintly.

FIG 11.—Uterine stem of a killed female *Br. bancrofti* from Mul. The outstretching larvae lie in conforming curves such as in free hookworm larvae result from the action of negative lateral thigmotaxis.





12



13



14



15

12—Killed female hamster living in the usual and attitude in branch node of Mad. The same section as the vagina and cervix, the uterine stem uterine branches with walls of green and red and the uterus.

13—Thick-walled section of uterine branch of killed female hamster in branch node of Mad. It contains, red-colored and green-colored embryos.

14—Cross section of the uterine stem of an already dead female hamster, seen [at] contains confined area of contrasting embryo.

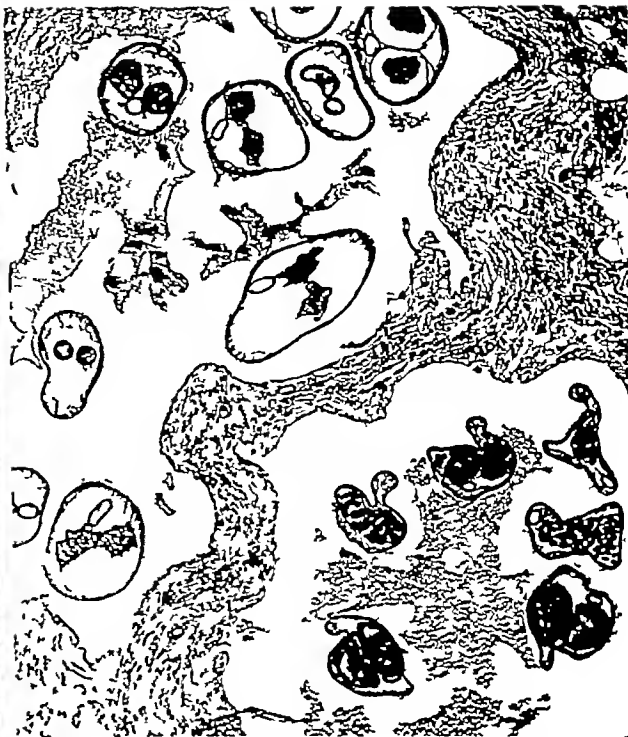
The uterine stem, at transversely and parts of it from the walls are of medium thickness (at least) but as of an already dead female hamster, the uterine stem are of medium length embryo better.



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FIG 16 —Transverse section of the ovaries of an already dead worm from Rod. Each forms a homogeneous mass enclosed in a defined membrane.

FIG 17 —Uterine coils of a worm presumably already dead, for there are missing the body walls and one of the uterine branches. In the coils, which in the material had not escaped from the host's tissues, these are in immediate contact with the worm's uterus and intestine. From Jam.

FIG 18 —The body wall of a killed worm on the left is folded; the uterine branches not being full enough to balloon it to rotundity, the body walls of the already dead worm in the right-hand loculus are distended to capacity by the mother worm's body fluid, which has crumpled the poorly staining worm viscera.



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FIG. 19.—Coils of an already dead *H. bancrofti*. The thick walled uterine branches contain outstretched embryos with poor definition of detail, the fluid in the body cavity has clotted and the clot has shrunk. The worm lies in dilated lymphatic of the spermatic cord of *N*.

FIG. 20.—An already dead female *H. bancrofti*. The body wall is calcified and cracked. Lies in hyaline osseous. From L. Day.

FIG. 21.—An already dead worm which had calcified throughout and had broken under the razor into sort of armour fragments. Lies in hyaline osseous. From Mal.

FIG. 22.—The uterine stem of *H. bancrofti* in peritarsus. A section in contraction of empty.

FIG. 23.—Another section of the same stem relaxed and showing in the section about two dozen eggs.



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FIG. 24—Uterine stem in an early stage of refilling. The section shows about twenty-four embryos which are uncoiling for the most part but with coils not intermingling. From Pen.

FIG. 25—Uterine stem with contents further developed than in Fig. 24. Its contents are uncoiling embryos that interlace in a pattern that defies counting. From Cox.

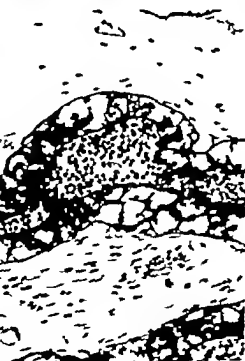
FIG. 26—Uterine stem in a stage of refilling yet later than that seen in Fig. 25. The larvae are outstretched and arranged in strands which themselves interlace. From Ken.



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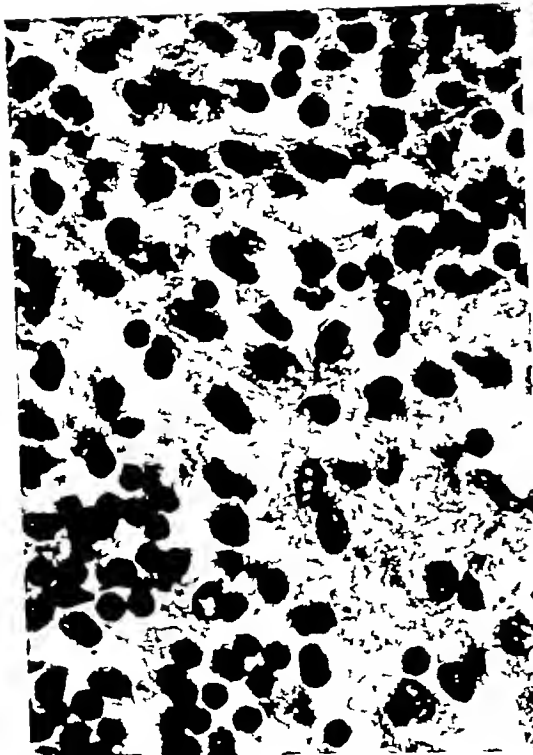


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FIG. 27.—Microfilariae clustered in an inter-nodular sinus of subcapsular lymph node of dog.

FIG. 28.—Flat-coated embryos clustered in an inguinal lymph node from dog.

FIG. 29.—Carbon particles sticking to the cells lining and to the fibrous crossing areas of lymph node of dog into whose lymphatic they had been injected upstream of this node. (The section is gift from Prof. C. N. DAVENPORT.)



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FIG 30 —Reticulo-endothelial cells fill an inter-mediary sinus of a lymph node. This field, free of microfilariae, lies next to that seen in Fig. 27 where the cause of the host's reaction is evident. From Mul.

FIG 31 —Parts of two lymph capillaries on the drainage route of a hydrocele. The upper capillary contains no microfilariae and its endothelial cells are normally flat with flat and deeply staining nuclei. The lower capillary has microfilariae in it; the endothelial cells are rounded in profile and their nuclei are round, lightly stained and nucleolated. From Sal.

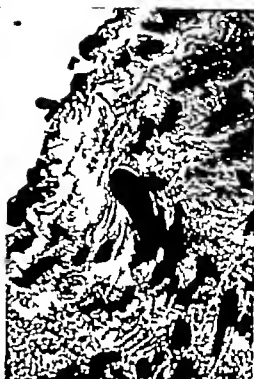
FIG 32 —A collection of giant and other macrophage cells in tissue round lymph capillary vessels that drain a microfilaria-containing hydrocele. These cells are destroying larvae that have escaped into the peri-lymphovascular tissue.



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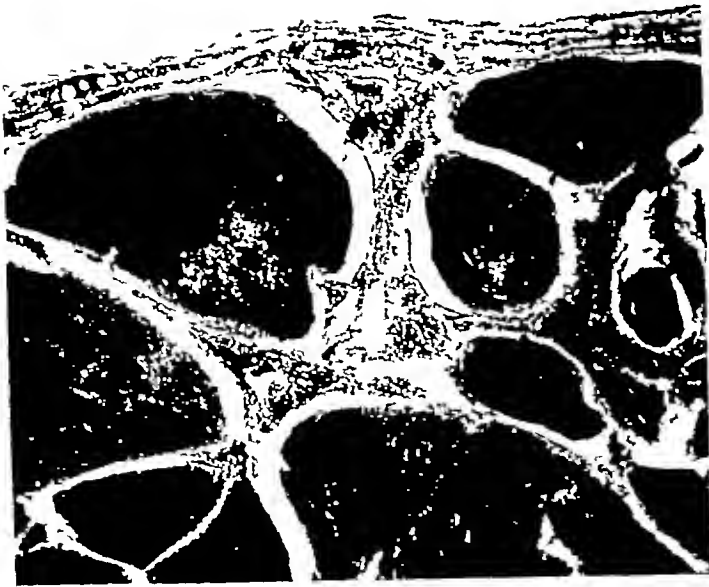
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FIG. 33—Subcuticular lymph capillaries are the dilated but still endothelium-lined forms subcuticular cystic space such as M. so packed here the first demonstrated that microfilariae though born into the lymph might fail to reach the blood. (From H.)

FIG. 34—A microfilaria sharply doubled on itself in a lymph capillary. (The new serial section the appearance is of two of the larvae are lying side by side in one capillary. (From H.)

FIG. 35—Lympho-vascular bed of the spermatic cord. The walls of the dilated vessels show great



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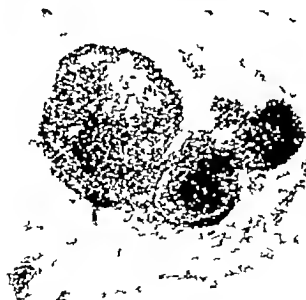
FIG 36 —A dilated and empty sinus of a lymph node of Rud. Its dilatation under raised pressure has snapped the fine baffle fibres that cross it the condition is manifest because illness of the host had sterilized the mother worms the node was no longer receiving microfilariae and in their absence the cells of the macrophage system did not conjugate

FIG 37 —Recent lymph clot which has shrunk away from the wall of a lymphatic vessel in the testicular region of Hol





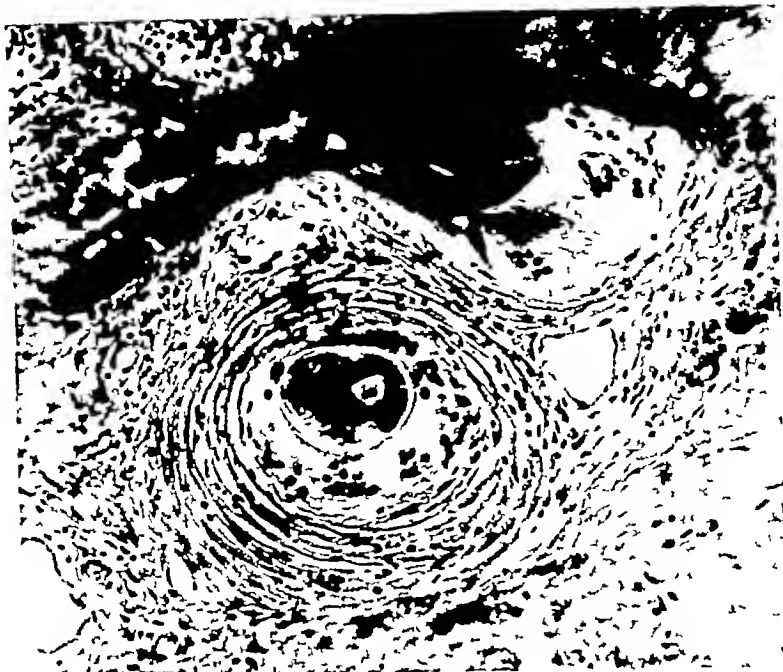
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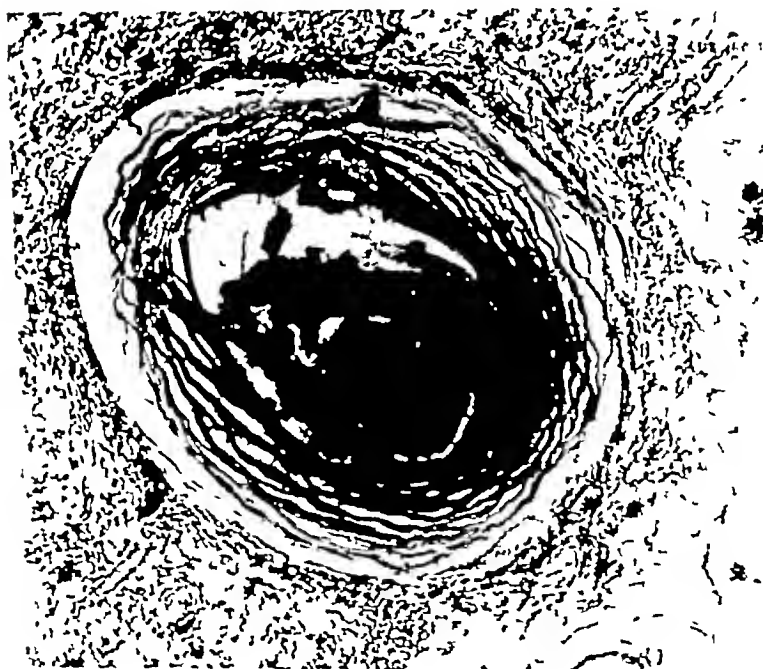
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FIG. 28 — A later effect of lymph thrombosis. The clot is organized into fine fibril which lead to longitudinal alignment. From Jan.

FIG. 29 — The actual remnants of lymph node have been squeezed into nodules separated and bound together by contracting fibrous tissue.



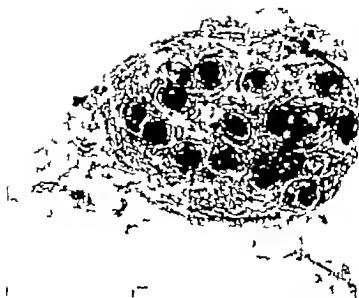
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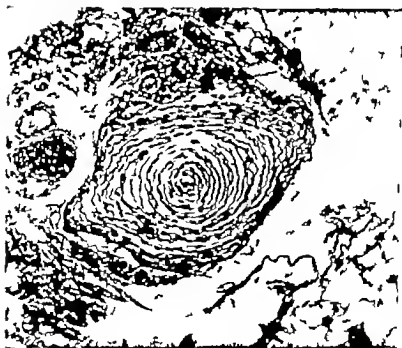
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Fig. 40. The structure in the making of a hyaline enamel. This one is in the capsule of a developing tooth. The innermost surrounding of a tooth is the pulp space, which is a mass of homogeneous hyaline material with large cells. The pulp space is in the peripheral part of the hyaline material is layered and the cells are arranged in a regular pattern. This is the stage when the enamel is formed. This is the stage when the enamel is formed. This is the stage when the enamel is formed.

Fig. 41. An example of a developing tooth. The tooth is in the capsule of a developing tooth. The innermost surrounding of a tooth is the pulp space, which is a mass of homogeneous hyaline material with large cells. The pulp space is in the peripheral part of the hyaline material is layered and the cells are arranged in a regular pattern. This is the stage when the enamel is formed. This is the stage when the enamel is formed. This is the stage when the enamel is formed.



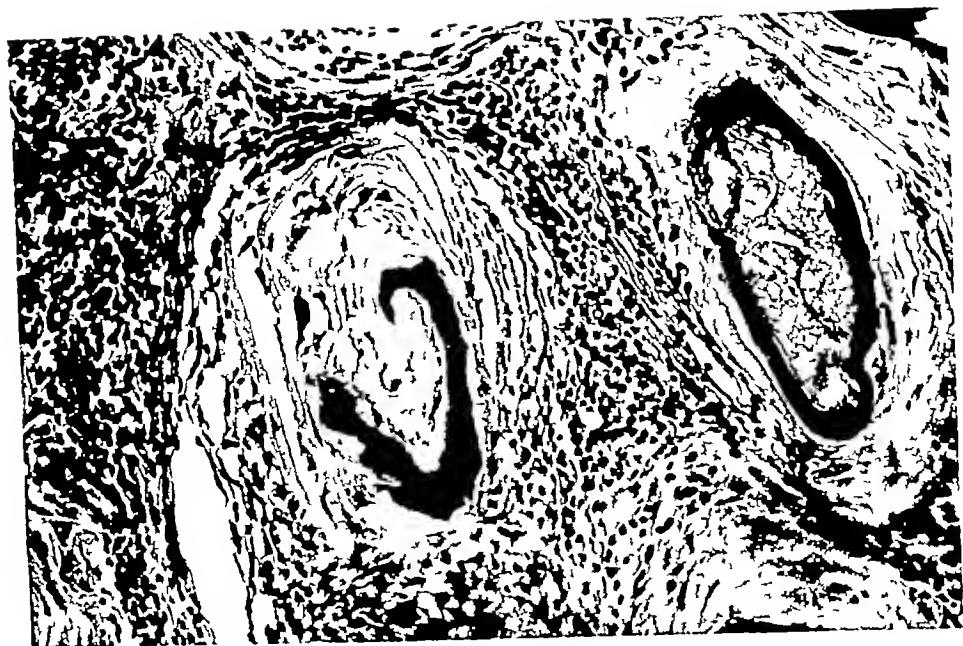
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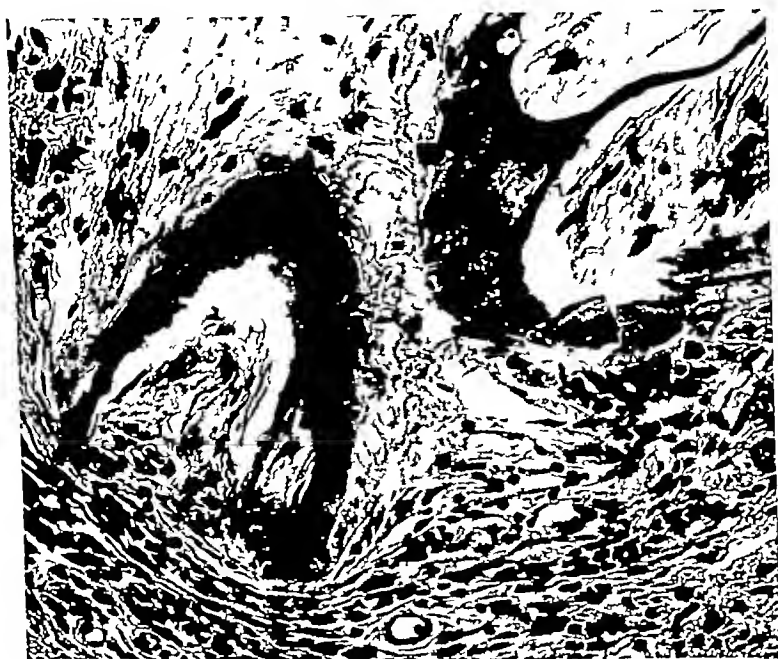
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FIG. 4.—A completely developed onion multiple onion formation surrounding many coats of dead calcified and disappearing cells.

FIG. 4.—A fine onion of delicate cells with small cells flattened cells on them. There is little of the onion round which it has grown.



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FIG 44 —Hyaline material invading a worm with a calcified body wall The material is fairly homogeneous inside and laminated outside the worm

FIG 45 —As well as hyaline material, lymphocytes and fibres are invading a calcifying worm

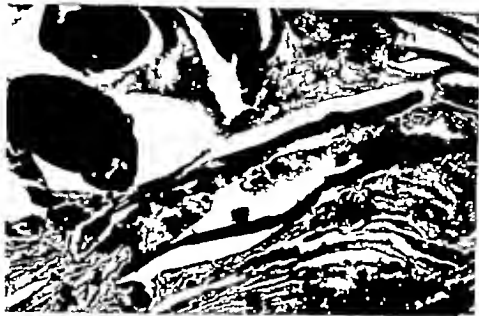


FIG. 45.—A superficially calcified  
 FIG. 46.—The calcified body all of  
 arm is covered by onion-like hyaline material,  
 arm is being broken up and invaded by cell and  
 fine fibrils.



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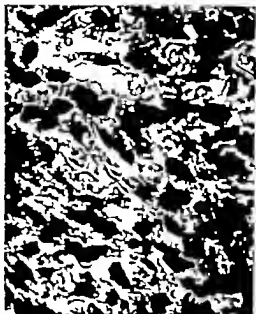
FIG 48 —One of the three adjacent coils of a dead worm whose body tissue is calcified. Invading host tissue has carried with it blood vessels. A capillary full of red cells lies inside.

FIG 49 —An almost normal and almost complete microfilaria in a section of the liver of Hol. The nuclei stain deeply and lie with fair compactness. In places the larval outline is slightly irregular.

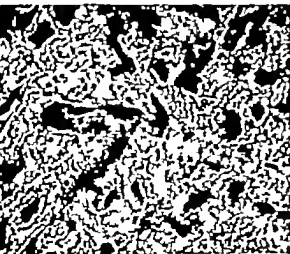
FIG 50 —A much degenerate long bit of a microfilaria living in a liver section from Mala.



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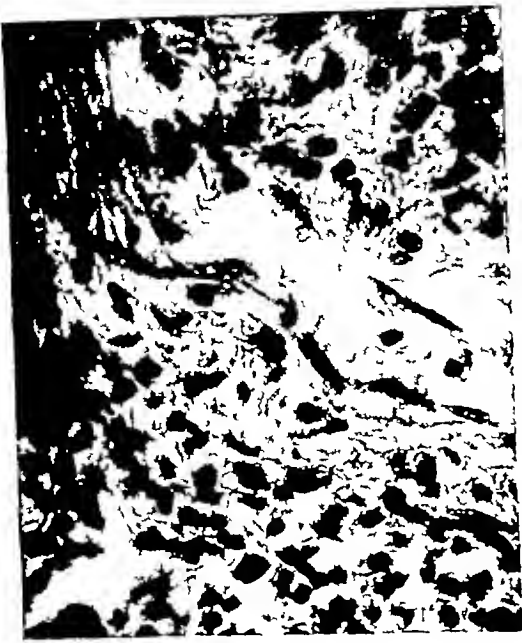


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FIG. 31.—Two macrofilariae in hist. section. One is in good condition—the other markedly degenerate.

FIG. 32.—Part of degenerate macrofilaria with very few nuclei in section of spleen from Mule.

FIG. 33.—A markedly degenerate macrofilaria bent on self with unusual sharpness in the spleen of Horse.



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FIG 54 —A long bit of the tail-end of a microfilaria whose outline is irregular and its nuclei scattered and faint, from the spleen of Hol

FIG 55 —Two microfilariae in a section of an adrenal from Mul. One is in good condition, the other degenerate

FIG 56 —Bits of a killed and an already dead microfilaria lie close to one another in a pulmonary alveolus of Hol



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## 7 SECTIONAL SUMMARY

Theories on the mechanisms that underlie microfilarial blood tides have severally been considered and summarized. They were all put forward without or with insufficient controls. Control of my own theory has now been made possible by study of O CONYOR's great material. The findings are set out in the Sections that follow

## SECTION II

THE RISE OF THE MICROFILARIAL BLOOD TIDE  
THE MECHANISMS THAT UNDERLIE IT

## A. INTRODUCTORY

Evidence is here offered that in the periodic bancroftian infection two opposing forces shape this chartered tide in the systemic circulation by synchronized peristaltic parturations the mother worms throw their young into the lymph stream by local macrophage system activity in the lymphatic tract and lungs of the host the young are subject to destruction before they reach the systemic circulation their complete destruction means no tide, their partial destruction a modified tidal chart.

1 POINTS TO BE ESTABLISHED FOR CAUSATIVE CONNECTION BETWEEN  
MATERNAL UTERINE STATE AND MICROFILARIAL BLOOD TIDE.

(1) Periodicity persisted when the material was got. (2) Only killed adult female worms have been used. (3) All these worms got at the same time have shown the same uterine state. (4) Uterine states of killed worms got at different hours are those that would be caused by synchronized periodic uterine emptying and steady refilling. (5) The phases of the microfilarial blood tide have corresponded in time to these uterine differences, after allowance has been made for the rate of the lymph stream.

2. THE STRUCTURE OF ADULT FEMALE WORMS, KILLED AND ALREADY  
DEAD

## (i) THE COARSE ANATOMY OF THE GENITAL CANAL

With two exceptions, the tubular genital tract of the female *B. bancrofti* runs tailward without loop or reverse from the vulva near the head end to the tail. It is at first single, all of this portion being commonly named vagina,

killed means that worms, adult or larval, were alive when the material was got and fixed already dead, that they had died before this was effected.

yet it is made up of two parts, structurally distinct. The vagina proper, that nearest the vulva, is a muscular tube with two coats, the outer taking the eosin stain and showing concentric fibrillation, and the inner having haematoxylin-staining nuclei, it is habitually closed or nearly closed with an empty lumen and at most hours evidently acts as a long and effective sphincter. The second part of this single portion of the genital tube is the uterine stem. Its wall consists of a single layer of deep cells, both nucleus and cytoplasm predominantly taking the eosin stain. At its headward end it narrows as a cone to join the narrower vagina, and at its other it divides into two uterine branches with the same general structure as itself. These branches then run on tailward side by side with walls lessening in thickness, and each continues as an ovary, at first tubular but solid at its distal blind end. The first exception to the steady tailward course of the genital tube is a complete loop made up solely of vagina, solely of stem, or of their junction. It was perhaps a loop consisting of stem in whole or in part that COBBOLD (1879, his Fig. 39) described as a "vaginal pouch crowded with embryos". The second exception is the doubling back on themselves once or twice of the ovaries close to their blind ends, so that a single transverse section of the worm shows them cut four or six times. The whole tract is then rather longer than the distance from vulva to anus.

#### (II) FINER ANATOMY OF A WORM KILLED BY THE FIXATIVE

In a killed worm (Figs. 11, 12, 13) the ovaries are cylindrical and finely granular with eosin-staining nucleolated vesicular nuclei round which the ovarian substance forms the cytoplasm of angular ova. In cross-section of the ovary the ova radiate daisy-like from the axis, while a continuous rim of ovarian substance persists after they have separated from this. As an ovum subdivides the nuclei become smaller, increasingly take the haematoxylin stain, and are so sited that, as the embryo becomes vermiform and they take station along its axis, they show up the embryo's attitude. This is at first that of a convoluted, and later of a flat, coil. On the way to its full outstretching comes a stage when the embryo is bent once on itself, all outstretched larvae in a stem evidently undulate in unison, just as do free hookworm or strongyloides larvae when by negative lateral thigmotaxy (LANE, 1933a) they form waving tufts projecting from some surface.

Conspicuous characters of a killed worm are a detailed clarity of structure and a sharp outlining of individual intra-uterine young, which, at the same uterine level, are at much the same stage of development. When advanced pregnancy has filled the stem with outstretched embryos, the packed columns of their axial nuclei are marked while the body fluid stains poorly or not at all. In uterine branches of medium thickness the larvae are in the flat-coiled stage.

## (iii) FINER ANATOMY OF A WORM DEAD BEFORE BEING PUT IN THE FIXATIVE.

Changes in a worm that has died or is at the point of death may be overlooked unless the living picture is fresh in mind and since a worm may die at any stage of pregnancy the intra uterine contents of one that has recently died may differ from that of one in the same material that was killed by fixing, and this may lead to wrong conclusions. There is need, then, to clarify this point even at length. Commonly all unborn stages in a worm already dead show diffuse nuclear staining and an embryo in the stem has a poorly defined outline. Later (Fig 14), the stem's contents lessen further in definition and may seemingly be made up of young at different stages of development. In Fig 15 one thick uterine branch contains obscurely fibrillar matter the other is full of granules separating a few embryos more tailward, the contents are confused and dimly outlined, and in each ovary a limiting membrane encloses a fairly uniform mass (Fig 16).

Another change was once seen. A worm had lain at the edge of a tissue block and had, it seems, partly escaped from this before being fixed and killed. The body wall was missing (Fig 17). It had not been floated off during mounting, for in the coils within the host's tissues, these tissues were in direct contact with the worm's viscera nothing intervened so the body wall had been dissolved presumably by the host's tissues and during the host's life. But the solution went beyond this, for in paired parts of the genital tube one branch was missing. The conclusion reached has been that like the body wall one uterine branch was unduly fragile (or the other unduly resistant to some dissolving agent, and remained in the tissue till this was fixed). Such solution without cellular reactions suggests a possibility to be aimed at and hoped for in treatment.

Another change was also seen once. The body-cavity fluid of an already dead worm in Pen. (Fig 18) is clearly under raised tension, for the body-wall is ballooned out and thinned, and the intestine is pressed in and its wall folded. This change is like that noted by MAC ARTHUR (1933) as one that follows the death of *Cysticercus cellulosae* in man in that infection subcutaneous cysts—as time passes—become evident to eye and hand and the raised tension further shows itself by the way in which a dead larva is shot out on cutting into the cyst.

This change in the already dead worm, in Fig. 18 is made more conspicuous by contrast with the state of the killed worms on either side of it. Their body walls are wrinkled and their uteri are only partly filled with young. The contracting uterus had, so to speak, sucked in the body wall and uterine refilling from the ovaries had not had time again to distend it before the worm was killed by the fixative.

This could be stated with certainty of the thin-walled sections containing early and late eggs with less certainty regarding the thick-walled portions of the branches which are less easily distinguished from the (two) single and thick-walled uterine stem.

Another and unobvious change in the body cavity fluid is its clotting (Fig 19) It is presumed to have followed the parasite's death within the living host, for though his tissues stain sharply, the staining of the intra-uterine larvae is ill-defined, as in other parasites that had so died

A change that may follow a worm's death is calcification, in sections showing first as dark haematoxylin staining often with cracks (Fig 20) of the surface or deep layer of the body wall Later, the whole worm may be involved and under the razor it may break up into pieces, like bits of a suit of armour (Fig 21)

#### (iv) MICROSCOPIC APPEARANCES OF FREE YOUNG, KILLED AND ALREADY DEAD

A description of these has been anticipated in considering the theory of POYNTON and HODGKIN (1938) They will again come under consideration when dealing with the fall of the microfilarial blood tide

### B SYNCHRONIZED, EXPULSIVE, EMPTYING PARTURITIONS PUT THE YOUNG INTO THE LYMPH STREAM

Expulsive uterine emptying implies in turn a full uterine stem, its emptying by peristalsis, and its refilling from the ovaries Evidence for this sequence is offered in the varying states seen in the uterine stem, and it is controlled, first by the finding free in the host of these less mature larval stages which are absent from a full stem but are present in parts of the full branches, and second by a distinctive siting of young free in the host's lymphatic system Evidence is next offered that in the periodic infection these parturitions are synchronized It follows that death or sterilization of mother worms will modify or annul the tide's rise

#### 1 EVIDENCE FROM THE VARYING UTERINE STATES

##### (i) THE FULL UTERUS

A nearly full uterus is the state seen in all worms from Mul (Figs 11 and 12) In the ovaries eggs are forming axially, in the thin-walled sectors of the uterine branches the eggs are early and eosinophil, in those with rather thicker walls they are multi-nucleated and haematoxylin staining, while further headward are mingled convoluted and flat-coiled embryos, in sectors with walls of medium thickness are flat-coiled and uncoiling embryos, in thick walled sectors and in the stem are outstretched "parallel-curving" embryos with some infertile eggs In the stem's cross-sections the number of outstretched embryos in ten unselected slides averaged 143.4 (90 to 253)

Plump stems that are full or nearly full and cones that are not, while all the young in the headward sectors of the uterus are outstretched as they are

in the blood, are the conditions to be expected were expulsive parturitions almost due, or had they begun with peristaltic emptying of the cone.

#### (ii) THE EMPTIED UTERUS.

The uteri of all killed female worms have in Rod. s. sections been emptied of all those outstretched forms that are seen in the nearly full headward sectors in Vul. This was reported thus by O'Connor and Hulse (1932) "In the uterine tubes of each of these worms the condition of development is identical. The uterine tubes contain ova. No differentiation of the ova into embryos is observed in any of the healthy specimens." But not only are the intra-uterine young immature, they are few and this is further evidence for expulsive emptying parturition.

Ny fifty sections show the cone twice, the stem seven times, and the thick branches thirty three times. The cone is empty the stem is either contracted and empty (Fig. 22) or is relaxed with about twenty four eggs in its transverse section (Fig. 23). Parturition is peristaltic.

#### (iii) THE REFILLING UTERUS

Various stages are seen in sections from Pen. Cog. L.Q. and h.a. In Pen. (Fig. 24) transverse sections of the stem show 0 to 24 embryos, and these are in the convoluted, flat-coiled or uncoiling stages, none being outstretched. The few and spaced young lie in a homogeneous material faintly haematoxylin stained. The whole looks like a honeycomb with one young form in each cell. In Cog. (Fig. 25) the stem is filled with intertwined curving larvae, having no particular alignment and so defying counting, but filling the cavity. In h.a. (O'Connor and Knott 1938) further outstretching and, so it is held, negative lateral thigmotaxy (Lowe 1933a) have formed larval sheaves that cross the stem in different directions (Fig. 26). Complete outstretching must bring all parallel-curving larvae in a stem (because it is narrower than their length) into a single sheaf that lies in general parallel to the stem's axis—and intra-uterine development, will have gone full circle to the state seen in Fig. 22.

#### (iv) THE UTERINE STATES SUMMARIZED

There is a stage in which the uterine stems and the headward parts of the uterine branches are filled with outstretched embryos lying on the whole parallel to the uterine axis. Uterine peristalsis sets in and these embryonic forms are expelled, their place being taken by young that had lain further tail-ward and that from eggs had developed successively into convoluted, flat coiled and uncoiling and finally into outstretched embryos the last interfacing at first singly then in sheaves and at last all being arranged as one sheaf aligned in the uterine axis and filling with outstretched young the headward parts of

the uterus, and the cycle of pregnancy and parturition is completed. These conditions are not those of more or less continuous parturition, they are those of expulsive emptying parturitions followed by uterine refilling from the ovaries.

## 2 EVIDENCE FROM THE YOUNG FREE IN THE HOST

### (1) THERE ARE FREE PREMIFILARIAL YOUNG

The finding of pre-microfilarial young free in the host is old, new knowledge gives it new implications. Over 60 years ago MANSON (1883) reported finding such young free in the host, for in his Case XXV he pricked vesicles in a lymph scrotum, and in the escaping fluid found both "[micro-] filariae and abundant active embryos struggling vigorously to stretch their chorionic membranes." In his Case XXIV he aspirated varicose lymph nodes and again recovered microfilariae and embryos still within the egg-membrane. BAHR (1912, page 67) confirmed the latter finding by aspirating six lymph nodes and by recovering microfilariae from all and premicrofilaria forms from two.

Were the aspirated young free individuals or were they still intra-uterine? Those MANSON got from vesicles were clearly free, for MANSON could not have overlooked a female worm lying in a transparent vesicle, so were those got by node puncture—unless one accepts the remarkable odds that MANSON once and MANSON-BAHR twice out of six times had the luck to run the point of the needle into the uterine stem or branches of a worm and, presumably, to hold it there while aspirating.

As has been noted, MANSON inferred that a worm may live in man for 32 years and that during that period it would, and did, at times miscarry, he held that the birth of premicrofilarial young was an abnormality, for his view he offered no control, indeed, it appears that the conditions of his practice in China precluded this. O'CONNOR's wealth of serially sectioned material enabled him to institute a control, part of whose results, in the slides I have had the benefit of examining, are seen in the table (page 732). The material brings out these points:

- (1) If lymph node material has contained any free young, it has always contained premicrofilarial young. The material is considerable, and this association invariable, the inference that the condition is one of normal labour seems inescapable.
- (2) In different material the percentage of slides that contained a particular developmental stage has greatly varied. So a statement that a certain premicrofilarial stage is absent from any material that contains young invites scepticism, unless the number of sections adequately covered has been considerable.
- (3) Premicrofilarial forms born in the host do not necessarily die at birth (a point not included in the table), all the material showed both killed

and already dead young in all their stages. "Premature" young do not die at birth, and while they live their development presumably goes on. This conclusion finds confirmation in lymph nodes from Dou, removed around noon during a hernia operation. The material of host and parasite equally lacks definition. It had evidently reached O'Connor imperfectly fixed. He examined several hundred sections and commented to me by letter on the fewness of premicrofilarial forms and the degeneracy of microfilariae. In my own eighty-eight sections I have found only four of the former (flat-coiled embryos three, degenerate egg one) in marked contrast to the numbers (table page 732) in slides from Rlc. and Cog from whom also material was got about noon and the condition is that which would be found had the unfixed young continued to develop and the unfixed macrophages continued to damage them at all their stages.

The evidence, then, is that many young are born while still coiled within the egg membrane, that after their birth they may rupture this and presumably escape from it. As Maxson pointed out, the active movements and small cross-section of living microfilariae are likely to help them to get through channels narrower than those which held them back while they were still coiled. But if embryos born in the flat-coiled stage hatch later they will in these conditions reach the blood tardily if they reach it at all.

Now evidence is offered below that the rise of the microfilarial blood tide is due to synchronization of those emptying parturitions above described. In that case the length of the crest of the charted tide will depend on at least three things

(1) The time taken by female worms to empty their several uteri.

(2) The differing times taken by microfilariae after their birth to reach the blood, even though born simultaneously in all parts of the body, for they have different lengths of the lymph tract to traverse and different numbers of delaying hazards to surmount.

(3) The extra time needed by young born within the egg membrane to burst this and to become microfilariae before they can get past these hazards.

These are matters of varying weight that need sifting when correlating the hour of synchronized parturition with that of the rise of the microfilarial blood tide.

## (ii) THESE FREE YOUNG HAVE A DISTINCTIVE SITING.

### (a) In by-passing lymph capillaries

Material got at necropsy from A, and sent to O'Connor, consisted of a testicle and 6 inches of attached spermatic cord. At neither end of this length of cord were there microfilariae in the lymphatic capillaries, they were limited

For this reason it finds no place in the table

to the middle sector (O'CONNOR, 1931), but here the weight of infection is in parts very heavy, for example, nine in a single high-power field. A microfilarial distribution confined to lymph capillaries in a limited stretch of the cord, and most numerous in the central parts of that stretch, would result from a short expulsive parturition by their mother. As noted (Section I) these by-passing lymph capillaries are so narrow and they enfold microfilariae so closely as to have been taken for their sheaths. That same narrowness would have prevented eggs and coiled embryos from even gaining entrance into these bypasses, they must have remained in the wider vessels with their brisker current, and this had evidently already carried them beyond the removed stretch of cord into the abdominal lymphatics or further.

### (b) *In lymph nodes*

In nodes the siting of microfilariae and premicrofilarial young may be as patchy as that first noted, their distribution being such as would be found did expulsive emptying parturitions force the young into the lymph stream over a limited time only.

In Mul (Fig 27) a group of microfilaria lies in a dilated sinus, another section from this node shows in a single field some microfilariae, at least ten flat-coiled embryos, bodies that are judged to be empty egg membranes (Fig 7), and thirteen or perhaps fourteen degenerate eggs, yet another section has in one field one flat-coiled embryo and six eggs, with microfilariae and flat-coiled embryos close by, a fourth section has in it fifty-nine flat-coiled embryos, ten being seen in one immersion field (Fig 28), while eight eggs lie in one such field of another section, with microfilariae and flat-coiled embryos just outside it.

From Hol a section of a preaortic node at diaphragm level shows one microfilaria and a cluster of seven flat-coiled embryos, five of them in one immersion field, four showing two coils and the fifth three. One section from a thoracic preaortic node shows not less than twenty-one flat-coiled embryos, at least twelve of them disintegrating in a lymph sinus.

From Cog a right inguinal node is cut tangentially, in about 2 sq. mm. of node tissue in four consecutive serial sections, each about  $10\mu$  thick, there are, especially in the cortical sinus, twenty-nine microfilariae, six doubled-up embryos, sixteen flat-coiled embryos and seventy-eight eggs.

In sections of nodes from Sal are many microfilariae, and where they are particularly numerous they lie in aggregations as striking as those in Mul, premicrofilarial forms are few and no aggregation of them has been made out.

In slides from Ric both microfilariae and premicrofilarial forms are few, and no aggregations have been detected.

*Comment*—When the young are found in numbers in bypassing lymph capillaries or in lymph nodes their siting is that which would be caused by



expulsive emptying parturitions, and some at least of it does not seem otherwise explicable.

#### (II) THE LEVEL TO WHICH PARTURITION EMPTIES THE UTERUS.

A main factor in determining from microscopic sections the level to which parturition empties the uterus is the observation that at any level of it all the fertile young forms in a killed worm are at much the same developmental stage (page 740). In Rod. the emptied uterine stems contain no young more advanced than the egg stage and there are not many of these—the last parturition had emptied the uterine stem and its branches at least to the level at which the full organ contains eggs whose contents have not yet developed to the vermiform stage. A control of the conclusion that emptying to this level is not exceptional is furnished by the developmental stages that were found free in other hosts.

It is the case that a lone egg (underdeveloped, degenerate and presumably unfertilized) will sometimes be found in a full stem, all of whose other contents are outstretched embryos. But as just noted, there are, in four consecutive serial sections from Cog., seventy-eight free eggs. This large number bears no relationship to the few that have been seen in sections of a full stem, nor has a full stem contained the flat-coiled or doubled-up embryos that are often found free. The uterus, in parturition, must have emptied itself to that level which in a full uterus is packed with segmenting eggs. Moreover all these free eggs appear degenerate, seemingly at that early stage they do not survive birth—in human terms prematurity is at the abortion stage.

Here again, is a commonplace in nature—a species survives by a profligacy which is greater than a habitual destruction of its young.

#### (IV) THE EXPULSIVE PARTURITIONS ARE SYNCHRONIZED

Evidence has been given that, in all O'CONNOR'S material from areas where periodicity holds, parturition has been by expulsive emptying. I have the impression, based on an admittedly inadequate examination of material from a non-periodic area, that the same holds there—if so, expulsive emptying holds for all races of *B. bancrofti*. It will cause a microfilarial blood tide to rise in one of two conditions

- (1) No host in periodic areas carries more than one fertile female worm, and her uterine capacity must be great enough to flood the blood as it is flooded.
- (2) If each host harbours more than one such worm, all come to labour at or about the same hour.

Regarding the number of mother worms, it is over 60 years since MEYER'S (1831) wrote: "They may be many" while O'CONNOR'S serial sectioning has established that parasitism by more than one worm is habitual and that in Ric. examination of serially sectioned inguinal and subinguinal nodes, of one side only revealed eighty in a periodic infection. So theory must conclude

that the rise of the microfilarial blood tide can be due to worm parturitions only if these are synchronized to take place at about the same hour. Evidence that they are is now offered.

Since worms may die at any stage of pregnancy or labour, and since the extent of development of the young varies with their positions in the uterine tract, an attempt to settle whether there is or is not synchronization of parturitions can be taken seriously only if there are compared not merely killed worms but the same part of the genital tract in the various killed worms in a host. The significant part is the uterine stem, for it is there that will be found the young that have at any moment reached their highest intra-uterine development and, by its singleness and its structure, it can be identified on examination of a single transverse worm section. But the essence of this line of reasoning lies in comparing with one another the uterine stems of the various killed female worms in one host whose microfilariae show periodicity. Examination of an unbroken series of sections can establish this, but equally certainly the same conclusion is reached if the materials from the same host have come from regions further from one another than the length of the stem. While the worm is about 18 cm long, her stem's length is only 2 cm. That distance limits certainty, though probability makes it even shorter in view of the characteristically coiled attitudes of the worms. The material to which these considerations have to be applied is as follows.

From Mul are sections of inguinal nodes from both groins. The condition of the uterine stems of worms from both sides of the body is the same. They are fairly full of outstretched embryos not packed to close "parallelism," though in the thick-walled branches (filled earlier from the ovaries and emptied later through the vulva), they are. These are different killed worms and their uterine states are the same.

From Kra the material was a lymph node mass measuring  $6 \times 3$  in. (about 15 by 7 cm.) which was serially sectioned in nineteen blocks, my sections from Blocks 5, 14 and 15 contained killed worms. O'CONNOR and HULSE (1935) showed such a mass of tissue and the numbering of the blocks into which it was cut. On these lines, five and fifteen were probably spaced and the worms, as the sections show, are as coiled as usual. In the stem sections the larvae lie in interlacing strands and their attitudes are the same in every block.

From Pen the material, a testicle, shows three adjacent varicose lymphatic loculi, each containing worm sections. In the two outer loculi all sections are of killed worms, in the interlying one all are of already dead worms (Fig. 18). Since there is no intermingling of killed and of already dead worms in any loculus, it is unlikely that there was a track between the outer ones, if there had not been, the sections in these separated loculi were of different worms. The contents of the stems of all killed worms is the same in all sections, namely, flat-coiled to uncoiling young. The inference that the worm sections in these two loculi are those of different worms is strengthened by material that came from a single node of Mul, in it two adjacent varicose lymphatic loculi contain worms, in one all the sections of these are of killed worms, in the other they are of intermingled killed and already dead worms. The contents of the stems of killed worms in both loculi are at the same development stage and, as in the material from Pen, the inference is that the sections of killed worms in these two loculi were those of different worms.

From the same groin of Dou there were removed (at intervals covering 1 hour) three lymph nodes, each of them containing killed worms. In all the stem sections from all three nodes the larvae have largely taken a position parallel to the stem's axis.

From Rod. three nodes were removed from one groin, two containing killed worms in the usual close-coiled attitude. The uterine stems in both nodes were in peristalsis and were empty or nearly empty the contents they had were eggs.

From Cog. only one node was removed. The stem or stems contain intricately intertwining young which during life would evidently have formed a squirming mass. The number of worms cannot be stated.

From Kra. the uterine stem of the worm contains crossing strands of larvae. There seems to be one worm only in the testicle.

From Sal. testicle shows worms that, from their appearance were probably dying when the material was got. The larval attitude is turbulent. The lymph node section contain no young.

From Mac. the node sections show no uterine stems.

This is the evidence in order of decreasing significance for the point under consideration. The uterine stems of all killed worms got from the same host at the same time have always shown the same stage of larval development. Sometimes they were certainly those of different worms sometimes this has merely been probable. There is independent control of this. MENOV (1935) found at an operation on the tunica albuginea three female worms all at the same stage of pregnancy. RAY (1936) had a like experience.

### 3. DO THESE SYNCHRONIZED EMPTYING PARTURITIONS CAUSE THE RISE OF THE MICROFILARIAL BLOOD TIDE?

These synchronized uterine emptyings furnish a reasonable explanation of the rise of the microfilarial blood tide. In future controls of this conclusion three points should have special attention. (i) The tides were present when material was got. (ii) the usual skin prick is enough to establish periodicity. (iii) the worms parturitions take place at an hour reasonably related to that at which the microfilarial blood tide rises.

#### (i) THE TIDAL RHYTHM PERSISTED WHEN THE MATERIAL WAS GOT

So regular is this rhythm that insufficient notice has been taken of its possible failure. Yet over 60 years ago MANSON (1881) showed that with fever it might fail, and BAIRD (1912) that in a non-periodic area microfilariae disappeared from the blood with inflammation whether accompanied or not by fever. Surprisingly it still needs insistence that, when trying to correlate worms synchronized parturitions with a microfilarial blood tide, there must be reasonable satisfaction that the tide persisted when material containing still living adult female worms was got.

#### (ii) SKIN-PRICK BLOOD RELIABLY DETECTS PERIODICITY

Blood got by skin-prick is no quantitative measure of the microfilarial content of freely circulating blood. This WARRINGTON YORKE and BLACKLOCK (1917) showed, when they compared blood got by skin-prick with that from the

Reproduced as Chapter 1 of his *Filariæ acquiræ hominis* (1883).

basilic vein; they found periodicity to be more marked in the skin blood, and concluded that larvae were detained in skin vessels

That they were in fact so held back was demonstrated by AUGUSTINE, FIELD and DRINKER (1936), they injected *Mf immutis* into veins of a bat and saw, in the blood circulating in the transparent wing membrane, microfilariae often so numerous in these capillaries as to occlude them, yet the young shortly regained the active circulation, bracing themselves against opposite walls of the vessel in which they were, and so passing either onwards into the veins, or back into the arterioles against the current until they were carried to unblocked capillaries. During the height of a microfilarial blood tide the microfilarial content of blood in skin capillaries (and presumably in those all over the body, including the lungs) will then be greater than in the larger vessels where it flows freely

It follows that skin-prick blood magnifies, and so detects with delicacy, any periodicity that may be present

#### (111) THE HOUR OF NORMAL SYNCHRONIZED PARTURITIONS BY FEMALE WORMS

Evidence above set out leads to the conclusion that in periodic bancroftian filariasis worm parturitions are of an expulsive emptying character and are synchronized. These conditions could result in the familiar rise of the microfilarial blood tide. A control will lie in a time relationship between worm parturitions and the tide's rise

The tide usually rises rather abruptly about midnight, but Charts III and V of O'CONNOR and HULSE (1935) show that the timing and shapes of these charted tides may vary markedly in members of the same family, and their Chart IV that like differences may hold for the same person on nights 2 months apart. It seems then that the getting of material to determine the hour of synchronized parturitions should follow at as short an interval as possible on the making of a blood-tide chart

##### (a) *The rate of lymph flow*

If it be the synchronized births into the lymph stream that cause the rise of the microfilarial blood tide, they clearly take place at an hour which enables that stream to deliver them into the blood in such numbers as will cause the tide to rise about midnight. To predict that hour and so control the matter needs a knowledge of the rate of lymph flow. My earlier conclusions which disregarded this link in the chain of evidence have misled.

Thus O'CONNOR and HULSE (1932) discovered empty uterine stems in Rod (Fig 22) from whom lymph nodes had been removed at about 14 00 hours. In assessing that report, I assumed (LANE, 1933b) that normal periodicity had been maintained till operation, which assumption implied a lag of some 12 hours between synchronized microfilarial births and the peak of the microfilarial blood tide. This conclusion is no longer tenable

Thus DRINKER and FIELD (1933) reported that trypan blue, injected into

the central end of an opened lymphatic trunk in a dog's leg, was present in the thoracic duct 30 seconds later and they pointed out that in 1895 THOMAS WINSKY showed that sodium salicylate did the same journey in 80 seconds. The suggested 12 hour lag in *Rod.* implies that a chemical substance will do in half a minute a journey which it takes a microfilaria about half an astronomical day to accomplish—hardly acceptable.

Accordingly the finding of empty uteri about noon suggests abnormal worm parturition in this lag—that this mechanism may fail has already been noted (page 746) and, as is stated below, other material got about noon shows no sign of recent parturition.

(b) *The getting and fixing of tissue to determine this hour*

Tissue for this purpose may be got at operation or necropsy. Operation allows of its being got and fixed at any selected hour and, accepting the conclusion that a normal microfilarial blood tide means normal parturition, it will, if extensively enough employed, disclose the hour of parturitions. Necropsy put through hours after death of the host, with the object of determining the worms' uterine condition at the hour when he died, will do so only if the parasite's reproductive mechanism comes to an immediate standstill with the host's death. Now the life of *Wuchereria* must depend essentially on the host's lymph flow and body temperature. After death his lymph flow continues, sometimes abundantly (page 726) and, in the tropics, his body temperature will not commonly fall abruptly—moreover if periodicity is due to synchronized uterine emptying by all healthy worms once in 24 hours, all the stages of pregnancy and parturition are compressed into that period and each stage lasts but a short time—accordingly the usual delayed necropsy will not necessarily show the worms' uterine state at the hour of the host's death—but immediate necropsy will do so with practically the same certainty as does excision during life.

But these procedures will display the uterine states, normal to a particular hour only if two conditions already noted have been fulfilled. (1) Parasites must have lived normal lives up to the hour when the material that contained them was got, and of this the only present clinical index is a persistent blood tide. (2) The material must at once have been properly fixed.

(c) *The hour of parturitions as indicated by O'CONNOR'S slides.*

(i) *Material got at operation about noon.*

O'CONNOR had found in lymph nodes removed between 13.45 and 14.28 hours uterine stems either empty or containing eggs only. As a control of my mistaken conclusion (page 747) that he had discovered the hour of normal synchronized parturitions, he obtained from various sources nodes that had been excised from seven other filarial patients between 11.00 and

14 45 hours and from one at 08 30 hours. In five of them my material is unhelpful in determining the hour of parturition, for my slides show the following conditions —

Material from Mac. has a killed worm but the stem is not cut in my slides nor are free young present, in that from Gar and Tor there are no killed female worms, in that from Jam. is a normally staining worm yet her body wall had in part disappeared during her host's life, so it may not be assumed that her reproductive life had been maintained normally till his operation, in that from Dou the tissues of both host and parasite have diffuse staining for the material was not fixed when it was supposed that it had been, so, as noted, it is without value in determining the hour of parturition since intrauterine larval development may have gone on after excision. Unexpected disappointments on this scale are significant of the care and patience required in future controls. O'CONNOR held on.

Material in my possession, already noted and obtained about noon from three other persons, does throw light on this matter.

Cog, while in good health, was operated on at 11 30 hours, for a right inguinal hernia and a right hydrocele, opportunity being then taken to excise large right inguinal nodes (O'CONNOR and HULSE, 1933, "First Case"). In all the sections of uterine stems of killed worms, the many interlacing larvae (Fig 25) form an intricate pattern, such as would result were embryos, having hatched in utero, become outstretched. Free young were abundant.

Ric was in good health in spite of having 1,058 microfilariae in 20 c mm of night blood, and right epitrochlear and right groin nodes were excised at 11 15 hours, in none of my sections is the stem proper cut, the cone is cut transversely and its narrow lumen allows short lengths only of larvae to be seen in any section, but their attitudes seem to be between those of Cog and Kra. Of the free young some, as in Cog, are in good and some in poor condition, while some are degenerate and becoming dissolved.

Kra, a youth of 17, was operated on at 14 43 hours for a swelling in the left groin of 11 years' standing, and a first attack of lymphangitis with a chill 5 days before his admission to hospital with a temperature of 102° F. This dropped to normal for good next morning, 3 days later there were microfilariae in the blood and in chylous fluid aspirated from the swelling. After 12 days of normal temperature spent in bed the enlarged nodes were removed as a mass measuring 13 by 5 cm. My sections show uterine stems, the larvae in them being outstretched and arranged as interlacing strands (Fig 26), they have no free young in node tissue but there are a few microfilariae in a lymph vessel close to the mother worm.

O'CONNOR's findings have this control. At his suggestion I wrote to MENON asking him at what hour the operation noted above (page 476) was done, and received a courteous reply, dated 27 6 35, putting it "between 11 a m and noon as the operations start early at the Reypura hospital."

#### (ii) *The findings in material got at necropsy at various hours*

Of these findings, those from persons dying about midnight are particularly helpful for the purpose in view.

Mul, aged 92, fell into coma from cerebral haemorrhage on 16 1 37, and remained unconscious till her death at 02 40 hours on 25 1 37. KNOTT did the necropsy 25 minutes after her death at St Croix, in the Virgin Islands, and sent the material to O'CONNOR in New York. The latter wrote to me "On 17th January she had a maximum of 220 microfilariae in 20 c mm of blood at night, and just before death of seventy-one in the same quantity." The material shows killed mother worms all of whose stems (Figs 11 and 12) are at least fairly full of outstretched embryos, it also shows many microfilariae free in a dilated sinus of a lymph node (Fig 27).

Sal., aged 68, was shot dead about midnight, necropsy took place at an unstarved hour next day. Testis with cord and hydrocoele intact, and inguinal lymph nodes were sent to O'Connor, who found four killed female worms on serial sectioning. He also received bits of spleen, liver and kidney. My own slides show that the larvae in the uterine stem have a turbulent arrangement while there are many free microfilariae in nodes.

Hol. died at 01 15 hours with heart failure, cyanosis, orthopnoea, oedema of legs and face and fluid in the body cavities. Necropsy was done 7 hours later. No killed worms are present in my ninety-four sections.

L.Q. (= A 293) died in Porto Rico at 00 40 hours, having been in hospital with cardiovascular disease for 5 days. Necropsy was done at 11 00 hours that day. I disclosed double hydrocoeles, and it was presumably for this reason that both testicles and cord were sent to O'Connor, the notes not mentioning hours of sleep or any blood examination made during life. I complete serial sections of the organs O'Connor found two worms, six being killed females. My sections show that both ends of the stem contain outstretched embryos lying in threads or strands crossing one another as in Kra. (Fig. 26).

Alor at about midnight, was shot several times through the chest, the bullets piercing heart, aorta, lungs and liver. Necropsy was done next day at 11 30 hours. The sections display no killed worms.

X was found dead in lodging house and necropsy done at 17 00 hours death was judged to have taken place between 05 00 hours and 11 00 hours. My sections contain no killed worms.

The tissue findings just summarized are such as would occur did synchronized emptying parturitions expel into the lymph stream a swarm of filarial young at an hour which (after the few minutes at most that are needed to traverse the lymph tract) would throw them into the blood at that at which its microfilarial tide normally rises. These findings show both the difficulties that have beset attempts to settle this doubt, and the way in which controls may meet them hereafter.

#### 4 THE SYNCHRONIZING STIMULUS

Whatever the mechanism that underlies the microfilarial periodicity of this infection, some stimulus must bring the larvae into the skin blood at night. If by the evidence set out here synchronized parturitions cause the rise of the tide that stimulus induces labour after passing through or originating in the host's body. Some 60 years ago MANSON (1883 page 57) defined what the stimulus is not. "As Dr MORTIMER GRANVILLE points out (Lancet 1882, i 314), it is not simply sleeping or waking that has this influence. Ingress [of microfilariae into the blood] commences hours before the usual time of sleeping and egress begins hours before the usual time of waking, and periodicity is maintained even though no sleep is indulged in for 2 or 3 days, or if sleep is continuous, or nearly so, for as long a time.

We are still ignorant of the stimulus, but in 1879 MANSON wrote to CORBOLD these words on microfilarial periodicity. This is striking and most suggestive fact, and in connection with it one might be tempted to speculate on the cause of the periodicity of malarial fevers. (MANSON BAILEY and ALCOCK, 1877 page 51.) Next year LAYRA discovered that protozoa caused these fevers and shortly afterward Italian workers notably GOLGI showed that malarial periodicity was due to synchronization of development and of what may not unfairly be called asexual parturition by parasites. S. CHEN (1939)

found, in canaries kept in lighted cages, that the peak of this segmentation settled down to that hour at which they were daily blinded by wearing a cap for the same 12 of the 24 hours, and that the same periodicity held whether the birds were allowed to feed throughout the uncapped period or only at its beginning. Again, alternation of high and low temperatures disturbed periodicity, yet if either extreme were continuous there was no disturbance.

Experiments on avian malaria have not disclosed such a stimulus as would cause periodicity in bancroftian filariasis\*, nor do these experiments bear directly on tertian and quartan malarias. In all these periodicities there are two factors: one brings the young to maturity\* in 24, 48 and 72 hours in the case of malaria and in 24 hours in periodic bancroftian filariasis, the other, that here considered, synchronizes their intramammary development and the mother's parturition.

The synchronizing stimulus remains unknown, nor is it known whether it acts through the male, bringing about coitus and impregnation at a certain hour, or through the female, for example by exciting parturition at a certain hour and thus leaving the uterus empty to allow another impregnation and the development of another brood of young. Presumably, one may reason from these malarial periodicities, if so the stimulus comes into being once in 24 hours but produces a response in parasites only when they are ready for it. In the periodic bancroftian infection all are ready for it every night, in its non-periodic form and in *Acanthocheilomonema perstans*, the response is imperfect or is wanting, in *Loa* infection the stimulus (if the same, as it presumably is) either gains acceptance at some other stage of intrauterine development, or the relative lengths of the parts of a 1-day development cycle differ.

HARLEY (1932) suggested that the stimulus is the saliva of that mosquito which is the normal host of the larval worm. He based his suggestion on fact and on supposition. The facts are that the larvae which have no microfilarial blood tide, which have one with a flood by day, or one with a flood by night, are carried respectively by insects that bite all round the astronomer's clock, that bite by day only and that bite by night only. The supposition is that if the rise of the microfilarial blood tide is produced by filarial parturitions, the injected saliva (or perhaps some substance the host produces from it) causes this tide to rise. MANSON pointed out the obvious time association between the flood of the microfilarial blood tide and the feeding time of certain mosquitoes, that the feeding causes the timing of the tide is without proof, but if so it should be possible to convert a non-periodic into a periodic infection by causing the host to be persistently bitten at night only by uninfected mosquitoes.

The synchronizing stimulus remains unknown.

## C LARVICIDAL MECHANISMS INTERFERE WITH THE RISE OF THE TIDE MICROFILARIAE THOUGH BORN DO NOT REACH THE BLOOD

### 1 HISTORICAL

Evidence for such an impeding mechanism can now be traced back to the observation of MANSON (1881) that he recovered microfilariae from the lymph vesicle while the man's blood showed none. He confirmed this observation later (1883, Cases XIV, XX and XXI). BAHR (1912) added a double control, from an enlarged lymph node he aspirated microfilariae, their sheaths and the worm's eggs, he watched an excised female worm passing young, in neither instance were microfilariae seen in the blood.

That this suppression is the work of cells of the macrophage system has been particularly shown by the work of TALIAFERRO and his colleagues. In his address, as retiring President of the American Society of Parasitologists, TALIAFERRO (1934) gave examples,

\* In the sense in which a human foetus is mature or immature



selected from the accumulating evidence, that the defence of the vertebrate against foreign bodies and against vegetable and animal parasites is essentially the same, and involves this system whose cells are derived embryologically from the mesenchyme.

SAXENA (1930) working on immunity against *A. pyostomatophila moris*, nematode parasite of the intestinal lumen of the rat, brought out the point that the reaction of these cells to this parasite infective larvae is something secondary, something that comes into being only after antibodies have effectively immobilized the larvae and such immobility may in practice be brought about either actively by repeated infections, or passively by injecting into uninfected animals sufficient immune serum.

Furthermore, they described in detail the development and morphology of macrophage system cells from three sources: (1) from the lymphocyte with two lines of development, one comprising polyblasts and macrophages, the other interpolating the monocyte between the lymphocyte and the first polyblast; (2) from reticular tissue cells variously named histiocytes in the skin, pericytes in the adventitia of blood vessels, septal cells of lung alveoli, stroma cells in the intestinal wall, and littoral cells in the sinuses of spleen, bone marrow, adrenals and liver, those in the liver being termed Kupffer cells only when they are visibly phagocytic; (3) from fibrocytes or fibroblasts which in the body can change into reticular cells and macrophages, though by most observers they are held to be specialized end cells.

TALLAFERRO (1940) took the matter further in his compilation of accumulated observations. At any spot where infecting organisms are detained there occur local production of antibodies and local recruitment of cells of the system. If enough antibodies are produced, and if they reach the blood, they can be detected in it, and acting from it they bring about the change in capillary permeability that accompanies inflammation, and this change allows of their diffusion back into the tissues of local areas of defence. In other words, whether reticulo-endothelial cells seen in an area have come into being locally or have entered that area from outside, they produce and excrete antibodies that oppose parasite worms, that enter the blood locally and that may leave it at some other spot where their action is needed. This, of course, concerns the destruction of filarial young in the lymph tract.

## 2. IMPEDING LARVICIDAL MECHANISM IN ACTION IN LYMPH NODES.

### (1) PHYSICAL AIDS TO THE ACTION

#### (a) *The structure of a lymph node.*

Pertinent knowledge has been put thus by DRECKER, FIELD and WARD (1934). After noting that its afferent lymphatic trunk breaks into a number of branches which pierce obliquely the capsule of a lymph node and open into the marginal or cortical sinus, they continue —

This sinus is not a channel but a large bowl-shaped lake bounded upon the outside by the capsule of the node and on the inside by the lymphocytic parenchyma. The lake is traversed by fibrous trabeculae and by blood vessels and, like all the sinuses, is crossed and re-crossed by a fine mesh of reticulum most important for the filtering function of the node. From the cortical sinus irregular but numerous cleft-like channels, the inter-mediate sinuses, pass between the masses of lymphoid tissue toward the hilus of the gland, where they unite with the cortical sinus to form the efferent lymph canal. As a result of numerous injections, it is our opinion that lymph flows as readily through the intermediate sinuses as it does through the cortical sinus. The entire arrangement, from the point of view of mechanics, is excellent for filtration. Lymph flowing in through

number of narrow channels, and under very definite head of pressure finds itself in a huge space with an enormous number of wide and irregular paths which lead to the hilus vessel. The flow is instantly slowed and the driving head of pressure practically lost. Not only are the sinuses in the node perfect straining chambers but the reticulum which they contain furnishes multitude of baffles which slow lymph flow and make it easy for the phagocytic cells in the reticulum to perform their function.

(b) *Filtration and sedimentation in a node*

In what immediately follows, "filtration" is used of the removal of suspended matter merely by size of pores, the sense in which MANSON (1883) used it in viewing a node as keeping filarial young from reaching the blood. He used the word "egg" for all stages before outstretching, he held that birth of eggs meant miscarriage, that a microfilaria being no wider than a blood corpuscle will pass through a node with no, or with trifling, injury, but that for an egg in a node's "solid parenchyma" things are otherwise, "for the imprisoned embryo has no power to aid its onward progress, but the egg lies like an embolus, passive, plugging the vessels and damming up the lymph. And this process of embolism, stasis of lymph, diversion of current into anastomoses, will go on until the whole of the lymphatic glands, directly or indirectly connected with the vessel into which the parent worm ejects her ova are rendered impervious, provided the supply of embolic ova is sufficient, kept up long enough, or renewed from time to time."

But this view is inconsistent with what is in O'CONNOR's abundant material. If any of it has contained microfilariae (that is, free mature larvae), it has also contained premicrofilarial stages. The birth of these latter is constant, that is normal. If it were the birth of these that caused progressive impediment to the lymph flow, then the parturitional life of the female worm is largely spent in giving birth to young in such a way as progressively to extinguish the species. Yet the species flourishes. It is the host's reaction to the young, in the various ways to be considered below, that impedes the passage of microfilariae to the blood to form food for mosquitoes.

(c) *Stickiness of sinus walls*

Diluted Indian ink, when run into a lymphatic trunk in a dog's hind leg, becomes deposited on the cell surfaces lining and on the fibrils crossing the sinuses of nodes into which the vessel drains, and finally blocks them. The striking appearance caused by a light deposit that does not fill and block sinuses (Fig 29) suggests to me that, apart from and perhaps as an aid to phagocytosis, there is a stickiness\* of the surfaces of these structures which has been a factor in bringing the particles to a stop. If so it may equally be a factor in detaining filarial young at a spot where the biological side of the mechanism may reach and destroy them.

(d) *Packing of node sinuses with host's cells*

The reasons that have been given against the belief that embolisms of still-coiled embryos can effectively dam the lymph current through a node

\* The condition differs then, from that in blood capillaries where carbon suspended in the blood stream, adheres to the sticky [inter-endothelial cell] cement without adhering to the exposed surfaces of the endothelial cells. (DRINKER and LOFFEL 1941, page 30)

are confirmed by the long search of a section that may be needed to find any larvae at this stage, when it is known that they are there. It is far otherwise with active cells of the macrophage system which so pack sinuses in which bancroftian young lie (Fig. 30) that the lymph current must further be slowed and microfilariae held up just where their destructors are most numerous. An occasional giant cell may be found among them (Fig. 8).

## (ii) MANIFESTATIONS OF THE SYSTEM'S LARVICIDAL ACTIVITY IN LYMPH NODES

The system's larvicidal activity in nodes that are receiving microfilariae is made trebly manifest. There is first the general inference, for which TALIA FERRO has been quoted (page 752), that at any spot where there is visible recruitment of the system's cells, there the system is locally active—there is second the observation just noted that there is conspicuous recruitment of these cells in nodes which contain microfilariae—there is third the observation that microfilariae in these lymph nodes may show the visible changes of dissolution (Fig. 8.) As to this last, O'CONNOR's material covers microfilaria-containing nodes from six persons. In that from one it reached O'CONNOR imperfectly fixed, for both the host's tissues and the microfilariae were degenerate, and the changes seen in microfilariae may not be accepted as having taken place during the host's life. In that from the other five the host's tissues show the normal staining in all but in all there are microfilariae in process of dissolution.

## 3. LARVICIDAL MECHANISM IN ACTION IN AND ROUND LYMPH CAPILLARIES.

In general, "when a lymph vessel enters a gland its endothelium under goes an abrupt transition from a merely passive mechanical function to powers of considerable activity" (HADDFIELD and GARROD 1934). But in this infection such a change in structure, and inferentially in function, may take place in lymph capillaries that contain microfilariae. In such capillaries (these drained a hydrocele in Sal.) the endothelial cells are no longer flat with deeply stained flat nuclei, but have become rounded with round lightly stained nuclei (Fig. 31) while of the microfilariae which they contain, some have been killed by the fixative, others were already dead when put in it. Moreover in the connective tissue round these lymph capillaries there lie degenerate microfilariae surrounded by lymphocytes, plasma cells, polyblasts singly or in groups, and giant cells (Fig. 32). These microfilariae might have left the capillaries passively or actively.

Passive escape is a reasonable result of the raised lymph pressure that often accompanies this infection—a feature that is considered in the next section.

Active escape of microfilariae from the lymph tract implies positive forward thigmotaxy (LANE,) this instinct, usually latent, must yet readily be

excitable, for it is by its urge that the species survives. When a microfilaria has been swallowed by a mosquito, it is this that impels it to pierce its way through its sheath which it has pressed against the wall of the insect's midgut and through that wall into the surrounding tissues, the cast sheaths, as O'CONNOR's slides show, being later passed in its droppings. I have not found this evidence of active escape in the slides from Sal, as is to be expected, for the sheaths would be carried off by the lymph stream, possibly beyond the length of spermatocord at O'CONNOR's disposal and even into the abdominal lymphatics. But premature and unnatural thigmotaxy does occur, as has been illustrated in Figs 1 and 2. Confirmation of this perversion of instinct would be easier did there come into general use a stain selective for the "chitinous," of which a cast sheath consists, for I may well have overlooked transverse sections of these inconspicuous objects.

#### 4 THE STIMULUS THAT SETS THE LARVICIDAL MECHANISM IN ACTION

The activity of macrophage cells that comes about from the presence of bancroftian young must result from some stimulus, and since this activity is common to and probably invariable in, these stages of helminthic infections which are cited in tissue fluid and lymph, all have probably a like stimulus. For the infective larva of *Nippostrongylus muris*, TALIAFERRO and SARLES (1939) have concluded it to be twofold. The primary stimulus is larval secretion and excretion, for in rats made moderately immune against this infection, precipitates lie beside those openings of the worm's body from which these products escape, and round the precipitates again are aggregations of macrophage cells, in hyperimmune rats the larvae themselves, the precipitates they produce, and the cellular collections they attract are all smaller than usual, and it may be assumed that the excretions that cause them are so too. The secondary stimulus is physical, with hyperimmunity, larval movements are slighter, do less physical damage and induce less cellular reaction.

#### 5 THE LENGTH OF THE LATENT PERIOD WHICH THE IMPEDING MECHANISM ENFORCES

BAHR (1912, page 67) showed that the mechanism which prevents microfilariae from reaching the blood is in action early in the infection, presumably it is so from the beginning. Accordingly, the latent period has two parts. The first is the time taken by a female infective larva to become adult and produce young, it is presumably fairly constant. The second part is the interval between the time that the first female parasite reaches maturity and bears young and the first appearance of microfilariae in the blood, and will vary with the hazards that lie between the mother worm and the systemic circulation.

Microfilariae have been found in a child of 14 months (ANDERSON *et al.*, 1924). As a measure of the latent period, this may err in either direction. It

may be too long, for an earlier examination of that child might have shown microfilariae it may be too short for the child may have become infected before birth. For *B. bancrofti* this latter is an inference a reasonable inference from observations on other helminths of which a summary was made by FOSTER (1935), the list including *Dirofilaria immitis*.

Prenatal infections by *B. bancrofti* would, presumably occur thus. Infective larvae from mosquito would bore their way into the blood of pregnant woman, would be carried by this to the placental site where, still keeping their thigmotactic urge they would force their way into the foetal tissues, and there during the child's intra-uterine or free life would grow into adults and produce young. In this case the latent period would be greater than the child's age since birth.

#### D THE LATER EFFECTS OF THESE LARVICIDAL MECHANISMS

There need consideration the mechanisms by which these ill-effects are produced and overcome. The effects arise from two interacting factors, excessive extracellular lymph protein and fibroblastic activity the fibrous formation that they induce around the lymph tract contracts and interferes with the further free flow of lymph through it however caused, the final effects are obstructive.

Histological evidence of raised lymph pressure resembles visual evidence for raised blood pressure. Vessels are dilated, and where they have muscular walls these are patchily thinned or thickened. The earliest evidence traced for dilatation goes back to MANSON's observation (1883 Case XXII) of skin vesicles that when ruptured or pricked, discharged microfilariae.

O CORCORAN's sections show these pertinent details. He received material removed at operation from Hut., namely vesicle-bearing skin over an old scar (Fig 33) the vesicles are in fact a network of dilated, but still endothelial lined, lymph capillaries. In the spermatic cord of Gal. such a local capillary network may be beginning to form. Fig 34 shows a double-up microfilaria thrust laterally by back pressure into the mouth of a bypassing lymph capillary the next serial section appearing to show pair lying side by side at all events, there is a marked lymph varix here with typical changes in the muscular coats of the vessels (Fig 35). In Rod. the female worms had ceased temporarily to bear young, so the destructor cells of the macrophage system no longer fill lymph node sinuses, and it is the emptiness of these that makes it evident that they are no longer crossed by baffle fibres (Fig 36) which must have been stretched beyond the breakpoint. Should stretching of a vessel, particularly of a capillary reach the bursting point and should microfilariae be thereby ejected into the tissues, they would cause the condition seen in Fig 37 so soon as their extravascular presence had locally recruited their destructors.

Clinically obstructive results of this back pressure, namely elephantiasis and hydrocele, are notable in this infection.

FOSTER (1937, 1937) points out the larvae of *Aedes taeniorhynchus* which entered pups in this way did not develop till after their birth, but that 10 to 40 per cent. of the larvae from infected mothers reached their intra-uterine pups and, after their birth, grew to maturity in them.

## 1 ELEPHANTIASIS

This conspicuous accompaniment of filariasis is itself accompanied by and is, presumably, due to an increase of extracellular protein in the lymph of the part concerned, and this, in turn, may be brought about either by lymphatic back-pressure or by disintegration of cells in the lymph. This statement has now to be justified.

The place of excess of intracellular protein in causing elephantiasis has especially been made manifest in DRINKER'S laboratory in Boston. Thus, blood capillary endothelium is "permeable to protein in both directions, though under normal pressure and osmotic relations protein that has left the blood is returned by the lymphatic tract alone" (FIELD and DRINKER, 1931). "Our experience points clearly to the fact that the principle function of the lymphatic system is to draw off extracellular protein. Lacking this drainage cellular environment becomes abnormal and fibrosis begins" (DRINKER, FIELD, HEIM and LEIGH, JNR, 1934). Finally, the last writers give these average percentages of this free protein in fluids of dog and man respectively: serum, 6.5 and 8.59, normal lymph, 1.43 and 0.49 to 0.69, elephantiasis fluid, 4.15 and 3.03. Elephantiasis fluid has then the high protein content which justifies the conclusion that it is this change which brings the condition into being.

That change may be caused by lymphatic obstruction, for workers in DRINKER'S laboratory produced it in dogs by effecting thrombosis in lymphatics and sclerosis of their walls. Both were needed, for, after thrombosis alone, regeneration of lymphatics with re-establishment of lymph flow is the rule, but as DRINKER and YOFFEY found, elephantiasis is more certainly established by repeated injections into lymphatics of crystalline silica suspended in a 2.5 per cent solution of quinine hydrochloride. Before an injection into a lymphatic, these vessels were made obvious by injecting between the toes of a dog a suitable dye and passing it on into lymphatic trunks by gentle massage, after which a quartz canula could be got into almost any lymphatic vessel (HOMANS, DRINKER and FIELD, 1934). Repeated occluding injections cause, in order, a transient pitting oedema, a permanent oedema, a brawny swelling, and finally elephantiasis with sclerosis of lymph nodes and lymphatic trunks. In established elephantiasis, skin and subcutaneous tissue show under the microscope marked fibrosis, oedema fluid between fibres, rare collections of lymphocytes, and many dilated but endothelial-lined lymphatics—a lymphatic network remains (DRINKER and YOFFEY, 1941). There is indirect evidence that the same change has been deliberately produced in man. For example, an annotation in the *Lancet* (1919, 1, 422) notes a report by Professor MERRILL, of Toulouse. Soldiers, by obstructing for days the circulation through a limb, produced lymphatic infiltration so great that it might not pit on pressure and might be permanent. Repeated or sustained lymphatic obstruction, deliberately effected, produces elephantiasis. In O'CONNOR'S material from bancroftian filariasis in man, the effects of the causes that produce it experimentally (and others) are seen.

#### 1 OBSTRUCTIVE MECHANISMS THAT OVERLOAD LYMPH WITH EXTRACELLULAR PROTEIN

The obstructive causes that in this infection overload lymph with extracellular protein are at least three-fold.

##### (a) *Lymph thrombosis*

Underlying the experiments that have produced elephantiasis has been the observation that lymph clots. A lymph clot that fills a vessel will obstruct the lymph flow through it and will add to the protein content of the lymph in the tributaries that have been draining through it, and that blockage will be a step on the way to elephantiasis. Such lymph clot is seen in O'CONNOR'S sections, either recent and shrinking (Fig. 37), or older and organising (Fig. 38).

What induces clotting call for further inquiry for lymph thrombosis has a protective side since it forms a "mechanism of fixation" barrier hindering spread of bacteria from an inflamed and infected area (BANCROFT, 1935), and such inflammation is well-known and unpleasant complication of bancroftian filariasis. It has not, I think, been considered whether some use cannot be made of lymph thrombosis to hinder the passage of microfilariae both into lymph nodes (so lessening the damage which they cause to them, and the serious after-effects which that damage produces) and their passage into the blood (so lessening the chances of adding to infection and reinfection including self reinfection). But this raises the matter of lymph embolism and the question whether the acute bronchitis which BAER (1912) found in Fiji to be associated with these stacks, may not itself have been due to lymph embolism.

#### (b) Reaction to microfilariae

As noted (Fig. 30) the presence of microfilariae in node sinuses leads to great accumulation in these passages of reticulo-endothelial cells. The fibrocyte is the generally accepted end cell of this series and is evidently the source of fine fibrillation seen in some nodes. A further step in this change is dense fibrotic feltwork which cuts node into nodules (Fig. 39) and whose contraction will further narrow lymph channels. The early accumulation of these cells, their development to the fibrocyte stage and the massive fibrosis this causes are probably the most effective and persistent causes of lymphatic obstruction in this infection.

#### (c) Reaction to adult worms

From O'CONNOR's material no support is given to the confidently made assertion that living adult worms may effectively block lymphatic. If they have always lain in loose coils in dilated sectors (Fig. 11) and with ample fairway between and round the coils. But round and within dead worm there takes place changes strangely if superficially recalling those of the promptly disposed of dead Phorob—chemical infiltration, enveloping and encasement.

Chemical infiltration is by calcium salts and may at first affect the body wall only. Round dead worm may be seen hyaline deposit with variously disposed reticulo-endothelial cells. In Fig. 40 this deposit is mainly homogeneous but with slight coarse layering peripherally or the layering may be more widespread but not yet complete (Fig. 41) or it may enshroud each separate worm coil (Fig. 42). In Fig. 43 the coarse onion formation has thinned to fine network round trace of the worm.

With this enshrouding goes eviceration. In Fig. 44 the coarse hyaline shroud has penetrated the body wall. In Fig. 45 fine reticulo-endothelial cells follow and in Fig. 46 t and small cells pass along within the calcified tube of the worm body wall. Fig. 47 shows worm section surrounded by concentric layers of fine fibrils in whose interstices are crowded small reticulo-endothelial cells. The calcified body wall of the worm has been broken through, some small and less small cells of this series are seen entering the worm and others have already taken the place of the worm viscera. In Fig. 48 bands of fibrous tissue surround worm section the ring of its calcified body wall is locally complete and is filled with fine areolar tissue of the host in which is conspicuous blood capillary endothelial lined and filled with red cells. Eviceration is complete.

#### (b) NON-OBSTRUCTIVE CAUSES THAT OVERLOAD LYMPH WITH EXTRACELLULAR PROTEIN

Lymph protein may be increased otherwise than by back pressure. DRUCKER and YOFFEY (1941) note that normal lymph contains few red cells and that they have never found them in numbers in lymph obtained by massage but active movement increases their numbers, typical experiments in dog giving these results. In quiescent lamb there was seldom any lymph flow in massaged limb it flowed but never contained large

Normal lymph has an excess of antithrombin, little thromboplastin, and calcium content corresponding to that of blood (DRUCKER and YOFFEY 1941)

numbers of red cells, in an actively moving limb there was at first an occasional red cell, after walking for 11 minutes there were 2,200 of them per c mm, after 13 and 27 minutes of running the figures were 3,600 and 13,600 respectively. In thoracic duct lymph red cells were also present in varying numbers, and though a search of some time might be needed before the first was found, they were usually fairly plentiful. If red cells are destroyed in lymph nodes, whether haemolymph or ordinary, extracellular lymph protein will thereby be increased, but their very presence in lymph seems to imply that blood plasma and its proteins must almost have reached it direct. Yet another source of extracellular lymph protein will be that destruction of microfilariae which was implicit in MANSON'S observations of 1881, and is manifest in O'CONNOR'S slides.

### (iii) THE RESTORATION OF LYMPH FLOW

Shrinkage of recent lymph clot reopens the old passage (Fig 37). Organizing clot that has filled a lymph vessel, perhaps by repetition of clotting, may show cleavages or may separate from the vessel wall (Fig 38). The homogeneous hyaline material that both enshrouds and enters dead worms splits concentrically and ends as a fine open meshwork round the place where a calcified worm lay. Round the body of a dead worm giant cells may lie in ranks, and one has been seen lying in a bay in the worm's outline, being evidently in process of dissolving its way in, a first step towards the worm's disappearance and, presumably, towards a reopening of the lymph flow through that vessel on which it lay.

## 2 HYDROCELE

As has been noted, when there is hypertension and stasis, or even reversal of flow, in lymph vessels, there is escape of microfilariae from them into the surrounding tissue. Round these that drain the tunica vaginalis reticulo-endothelial cells collect and there results a swelling. In Sal this extends to the mouths of these vessels, where they take origin from the tunical cavity and show in section as a papilla, capping this true tissue papilla is a false papilla composed of clotted lymph in which are entangled microfilariae mostly dead or dying and host cells in like state. The tumid tissue will compress the lymphatic capillaries that run through it, and the clot that caps it will still further interfere with the drainage of the tunical cavity. To these early forces that may produce hydrocele will, as time goes on, be added the contracting fibrosis that the reticulo-endothelial cells will cause and which will further compress the drainage lines. Whether, and if so by what means, adequate drainage is re-established the material has not suggested.

The presence of microfilariae in hydrocele fluid may have one of two causes. Either lymphatic obstruction has gone on to reversal of the lymph current, or the mother worm has an abnormal habitat in the tunical cavity. In the latter case the hydrocele fluid would presumably show microfilarial periodicity, in the former such a presumption is unjustifiable. The mere absence, in a particular person, of microfilarial periodicity in hydrocele fluid is necessarily without significance in attempting to determine whether the microfilarial blood tide is or is not due to periodic synchronized parturitions by mother worms.

## 3 SUMMARIZING COMMENT

Limb lymph, flowing normally, has a low content of extracellular protein, when its flow is obstructed this content rises and there sets in the fibrosis of elephantiasis. Lymphatic obstruction may be due to lymph clot, to enshrouding of a dead adult worm, to narrowing of the fairway of lymph node sinuses by aggregations of reticulo-endothelial cells attracted by the presence in them of bancroftian young, and later to the node fibrosis that these cells produce. But the content in lymph of extracellular protein may rise from at least four



other causes besides back-pressure in this infection lymph has in it more red cells than is usual. It has also in it microfilariae and cells of the macrophage system, some or all of which are degenerate and in dissolution, and dissolution of worm and host cells will presumably add to extracellular protein. Moreover where red cells have passed from blood into lymph vessels, plasma and its proteins can hardly have failed to do the same. With each type of lymphatic obstruction in the limbs there is seen in action a mechanism that re-establishes the lymph flow and so brings it about that elephantiasis by lymph stoppage must be as hard to effect naturally by this infection as it is experimentally by intralymphatic sclerosing injections.

Hydrocele is also the result of lymphatic obstruction and the mechanism has been traced above but one for re-establishment of lymph flow has not been detected in O'Connor's material.

### E. SECTIONAL SUMMARY

In using material to determine the cause behind the rise of the microfilarial blood tide, three essentials, it is felt must be observed. (1) there must be knowledge that periodicity was present when the material was got. (2) conclusions must be based on worms that have actually been killed by its fixing. (3) the uterine level selected for comparison must be the same, and this can be assured by using the uterine stem. The examinations that have been considered above have covered a large percentage of my 800 slides cut from twenty nine persons, yet slides from only seven of them fulfil the two last essentials. The first in the case of all of them is an inference held justified in the main.

The primary factor in the rise of the microfilarial blood tide is simultaneous, expulsive, emptying parturations. For their expulsive and emptying character the evidence is twofold. First their emptying has in fact taken place to the level at which the walls of the uterine branches have a medium thickness and at that level their contents when they are full are segmenting eggs. In control of this is the observation that in the host's lymph nodes there are present clusters of knot-coiled and flat-coiled embryos and of microfilariae, while only in restricted lengths of his bypassing lymph capillaries are there filarial young. All these are evidences of short expulsive emptying parturations into the lymph stream. These are synchronized, for in material got at the same hour from the same host the uterine stems of all killed female worms contain young at the same stage of development. With such rapid intra-uterine development (measured in hours and which we have no right to consider as ceasing at the moment at which, for legal purposes, we consider the host's life to have ended) it is necessary that examinations made for the purpose of determining the hour of parturations should use material got either at operation, or at necropsy done immediately after death, and in either case adequately fixed at once. On these conditions and taking into consideration the rate of lymph flow it appears that parturations take place about midnight. KNOTT making examinations about that hour

found a state of affairs that would be expected had he surprised the worms in the very act of labour. No one else seems to have looked for this evidence about this hour and no one has found it at any other. The synchronizing parturitional stimulus is unknown, it must be accepted by worms that have been bred in regions where periodicity holds and refused by those bred in places where it does not, the question arises whether it is the same stimulus that sets *Loa loa* in labour if here, too, the rise of the blood tide is due to synchronized (but daytime) parturitions, as presumably it is.

That there must be a secondary factor, one opposing the passage of newborn young from lymph to blood, MANSON'S early observations showed. It is executed by macrophage cells in lymph nodes, in lymph capillaries and in lungs. In the nodes it is aided by the slowing of lymph current which the structure and qualities of their sinuses bring about, and by their becoming crammed with these guardian cells when filarial young have entered them. Endothelial cells of lymph capillaries that contain microfilariae enlarge, and become active for some of these larvae are degenerating, microfilariae may escape from these vessels either by premature thigmotaxis or by rupture of capillary walls from back-pressure, and in the perilymphovascular tissues they, too, are set on by macrophage cells. The stimuli which call these cells into activity are larval buffetings, and larval secretions or excretions. The observed latent period between infection and the first finding of microfilariae in the blood has been 14 months, for they have been found in a babe of that age, though, if infection can be prenatal, the period of latency here may have been longer.

## SECTION III

### THE FALL OF THE MICROFILARIAL BLOOD TIDE THE MECHANISMS THAT EFFECT IT

#### 1 GENERAL CONSIDERATIONS

The rise of the microfilarial blood tide in periodic baneroftian infection is then due primarily to synchronized nightly births of microfilariae. But these tides do not get higher night by night. Of this there seem two possible explanations —

- (1) Each night an unknown mechanism releases from an undiscovered daytime haunt only enough microfilariae to keep the tides' peaks at about the same level in spite of wastage, and recaptures them next morning—yet the mechanism and the site of the dump remain undiscovered.
- (2) All, or practically all, microfilariae born each night are destroyed each day. What follows is the evidence which O'CONNOR'S slides furnish of their massive destruction by the visceral part of the macrophage system.

As HADFIELD and GARROD (1934) quote from ARCHOFF this system comprises the reticulo-endothelial cells of the spleen, lymph nodes, liver (Kupffer cells), marrow suprarenal cortex and hypophysis. Microfilarial destruction in the lymphatic parts of this system I have described (LANE 1937) in slides sent me by O'CONNOR during his lifetime. In the many slides he left me by Will this is amply confirmed. Before considering the mechanisms that effect the fall of the microfilarial blood tide, two points must be made or re-made.

Staffing his laboratory at Columbia University New York, O'CONNOR's team of skilled microscopists made, examined and, when desirable, marked thousands of serial sections. In any one of these a microfilaria will, from its shape, commonly be cut more or less transversely but with sections seen on this scale a considerable part of a microfilaria will occasionally lie in a single section (Figs. 49 and 50). Again, a destruction capable of causing the familiar sharp fall of the microfilarial blood tide must be extensive and rapid, and adequate search should make evident not merely this destruction but a periodicity in it the degree of larval degeneration varying with the hour.

My apposite sections come from lymph nodes, liver spleen and adrenals.

## 2. EVIDENCE FROM SECTIONS.

### (I) LYMPH NODE SECTIONS.

A lymph node can presumably get rid of filarial young whether they reach it by blood or by lymph. Their part in destruction of larvae that reach them by blood is undetermined—but is experimentally determinable.

### (II) LIVER SECTIONS.

From Sel. I have one slide only and have seen no microfilariae in it. From Mul., Mala. and Hol. I have respectively four, twenty-six and seven and in sections from all persons there are present both killed and already dead forms. For instance, among the more nearly complete and so more striking examples of larvae in O'CONNOR's material are those just mentioned, one from Hol. (Fig. 49), in which microfilaria, normal or nearly normal lies wholly or almost wholly in one section—the other from Mala. (Fig. 50) in which such microfilaria is degenerate. In Fig. 51 parts of normal and of degenerate microfilaria lie in one field.

This matter is otherwise illustrated in an analysis of the state of all microfilariae\* found on examining with an immersion lens and square-apertured eye-piece single unselected section from Hol. It covered 225 sq. mm. and was judged to be 10 $\mu$  thick. In it were detected 162 separate microfilarial lengths. thirty of them (18.5 per cent.) were classed as in good condition, for they had close column of deeply stained nuclei and an even body margin; the others showed different degrees of probable or certain degeneration up to that in which the nuclei were ghosts, while the body was either shrunken with wrinkled outline presumably the result of loss of larval substance or was bloated with its surface wrinkles evened out recalling the state of the dead adult worm seen in Fig. 18. Shrinkage and distension may be seen at different ends of the same larva and it seems that lessened depth of nuclear staining may precede or may follow nuclear scatter.

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\* But transverse sections of the larvae are not included, for these do not lead to easy determination as to whether they are or are not normal.

## CLAYTON LANE

## (iii) SPLEEN SECTIONS

In my fifteen sections from Mul the microfilariae have varied from a normal state to one of marked disintegration (Fig 52). In the three sections from Sal I have found all microfilariae normal, but a degenerate flat-coiled embryo lies in one. In eleven sections from Hol the state of some microfilariae has been good, of others poor (Figs 53 and 54), with marked irregularity of outline, scatter of nuclei and faintness of their staining. BONNE (1939) noted heavy eosinophilia and peculiar giant cells in a human spleen containing microfilariae whose species (*bancrofti* or *malayi*) was not determined.

## (iv) ADRENAL SECTIONS

My thirty-two sections from Mul contained killed and already dead microfilariae, sometimes both forms in one field (Fig 55). The single section from Hol shows a normal microfilaria and one whose nuclei are pale with some scatter.

BONE MARROW and HYPOPHYSIS — O'CONNOR's slides do not include such material.\*

## (v) KIDNEYS

In O'CONNOR's material I have detected no microfilariae that are not normal.

## (vi) HEART

I have not detected degenerate microfilariae in these sections.

## 3 SECTIONAL SUMMARIZING COMMENT

In O'CONNOR's sections of the visceral part of the macrophage system the extent of microfilarial destruction has been great enough to account reasonably for the fall of the microfilarial blood tide, thus in a single unselected liver section less than a fifth of 162 microfilarial lengths found in it could be classed as certainly normal. The relative share taken in this destruction by liver, spleen, adrenals, lymph nodes, hypophysis and other organs awaits yet wider investigations made at hours spaced twice round the common clock.

TALIAFERRO (1934) collected the evidence that the macrophage system provides the body's defence against dyes, particles and small parasites, vegetable and animal, to which list he and SARLES (1939) added nematodes. Should a call be made on that defence, it is met up to the system's capacity at the moment. Should this not be enough to meet the call, the system in technical terms is blocked, but as HADFIELD and GARROD (1934) note, its complete blockage never has been attained. In the ordinary, naturally developing, bancroftian infection it may be concluded that this defence is steadily built up to the extent which meets the parasitic birth-rate and destroys one nightly brood before the next is born. It is of interest to consider the degree to which blockage seems to have influenced the results of massive injections of microfilariae into man's blood vessels.

MURGATROYD (1933) injected 720,000 microfilariae into the blood, and in

\* NAPIER DAS GUPTA and SUNDAR RAO (1940) reported on sternal punctures made in the infection. Of ten smears made at night microfilariae were present in all, of forty-six made by day they were present in three. These writers felt that they had produced no evidence for microfilarial destruction in the marrow.

four examinations of it made during the next 21 hours recovered none. Though this man had served in the tropics, he had never had any signs of this infection, nor before their injection could microfilariae be discovered in his night blood.

KNOTT (1935) injected intravenously into eight inhabitants of St. Croix, Virgin Islands, U.S.A., numbers of microfilariae varying from 825 000 to over 2,000 000. The smallest number was injected into one whose blood was "negative to microfilariae" and who had no clinical signs of infection. blood films made 24 and 48 hours after the injection showed 0 and 2 microfilariae. The largest number was introduced into a man who had enlarged inguinal lymph nodes "chronic filariasis" of both testes and at the time of injection an average of 245 microfilariae per c.mm. of blood. in five samples taken 1 to 60 minutes later the corresponding average was 253. In all eight instances the injections of microfilariae in these numbers was followed by their complete or almost complete disappearance. St. Croix is highly filarial and at least one of those experimented upon was infected.

HAWKING (1940) injected into the blood vessels of four persons citrated blood containing 300 000 to 4 000 000 microfilariae. in two they promptly disappeared, in the two others 90 per cent. did so while numbers corresponding to the residue persisted, in one for about 10 days and in the other for more than 8, it being impossible in the latter at least to say whether periodicity did or did not exist.

Others have cited such experiments as disproof of the suggestion that the microfilarial blood tide is due to birth and death of microfilariae. But such experiments can neither prove nor disprove this matter. Indeed, their results are those that would be obtained had the macrophage system been equal to its greatly added burden. in MURRAY'S and KNOTT'S and in two of HAWKING'S cases but had it been blocked in HAWKING'S other two. This explanation fits the known facts. it can become proof only when adequate observation replaces inference.

Massive fibrosis of a lymph node goes with microfilarial destruction in it, and it has been suggested above (page 756) that some of that added extracellular lymph protein which induces fibrosis comes from this destruction. But cirrhosis of the liver and of other viscera in which microfilariae are destroyed, presumably in numbers, is no characteristic of this infection. For this apparent anomaly the following explanation seems reasonable. DRINKER and LUTTER (1941) report these percentages of extracellular protein in lymph and in obstructive oedema fluid in the leg of the dog: in normal leg lymph of dog the percentage averaged 1.55 (1.41 to 2.06 Table 18) in five examinations, oedema fluid from lymphatic obstruction of the leg averaged 3.6 (2.25 to 4.58 Table 44) in liver lymph of dog it averaged 4.32, and of cat was the same as plasma (there being no lymphatics inside a liver lobule) of lymph that leaves the spleen, the composition is unknown, and no one has described lymph capillaries within it or within bone marrow.

They do not not adrenal on this point.

The normal lymph of these viscera has then an extracellular protein content much higher than the normal limb lymph, yet in the viscera it produces no cirrhosis, and to this high normal content is being added in this infection (so the evidence suggests), more protein by the dissolution of microfilariae. But there is nothing to suggest that this further heightening of an already high normal content ought, if the facts are as stated, to be enough to produce cirrhosis, or that the absence of this pathological change throws any doubt on the visual evidence (Figs 50 to 55) that there is marked destruction of these larvae in the visceral parts of the macrophage system.

To the reasoning that periodicity is caused by daily birth and destruction of microfilariae, AFRICA, GARCIA and LAYCO (1935) raised two objections when they discussed their own findings in a man whose microfilarial population they estimated as 4,585,000. They write, "the theory seems unbiological since it would be a piece of great folly on the part of nature to permit such wholesale death, when, clearly, these microfilariae need to stay longer in order to have more chance of getting into the mosquito host." Yet few will deny that a biologist spoke with authority in these words: "A distinctive feature of organic evolution, marking it off from social evolution or inorganic evolution, is the relentless elimination. Relatively few of those that are born are ever themselves able to produce. As TENNYSON said, 'of fifty thousand seeds, she often brings but one to bear'" (THOMSON, 1925). An immense destruction of microfilariae (an occasional one developing in mosquito and man) is the measure of the obstacles which the species has to surmount if it is to survive—and it has survived just because it has sacrificed its young by the million. This objection, that the theory is unbiological, is not valid. The second objection by these writers is that a daily destruction of these great numbers must cause protein shock. Protein shock has not been a striking feature of the large single microfilarial injections cited above, and if a host's body becomes adapted to deal daily with large numbers of filarial young, is protein shock to be expected?

There takes place in the visceral macrophage system a destruction of microfilariae great enough, I suggest, to cause the fall of this blood tide, and objections made to this explanation I judge unwarranted. To what has been said earlier (Section I, page 723) to meet MANSON's difficulty in accepting microfilarial destruction as the cause of the tide's fall, this may be added. Since he wrote we have gained the conception of a macrophage system and the knowledge of the system's wide distribution through the body, and to this O'CONNOR's steadfast collection, preparation and examination of material have added visual evidences of microfilarial destruction in the system's visceral sites. In regard to the doubt as to the purpose (so MANSON expressed it) served by this enormous

\* "In itself the word Authority signifies primarily a statement or an opinion for the truth of which someone is prepared to vouch, more particularly an expression of responsible and competent opinion" (RAWLINSON, 1929).

daily mortality of microfilariae, I would today state the matter thus. Here is one more example of what is now known to be an everyday and all-day activity of the macrophage system in ridding a mammalian body of foreign matter including parasites.

Lastly it is reasonable to recall the experiences of VITERS and of STEPHEN MACKENZIE (Section I page 722) and to suggest this view. Elements in circulating blood are repeatedly passing through organs and tissues that contain macrophage cells. If there are microfilariae among these elements, and so long as they continue to circulate, they are time and again being brought into effective nearness to destructor cells. and the longer they circulate the more numerous will be these contacts and the greater the likelihood that the larvae will thereby suffer damage. By the evidence and considerations offered, microfilariae are born at night and are normally destroyed at least before the next day ends. Microfilariae in blood drawn at night will presumably have suffered less damage than any still present in it next day and may reasonably be expected to survive for longer in it than do those in blood drawn off next day.

## SECTION 11

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### A LARVICIDAL MECHANISM IN THE LUNGS

O'CONNOR'S lung material has come from Vul. and Hol. I noted (1937) that in my slides from Hol. I found only normal or nearly normal microfilariae. The larger number of sections that has come to me by O'CONNOR'S Will shows advanced destruction of some microfilariae in both persons (Figs. 55 and 56) while in Vul. an already dead flat-coiled embryo has been cut across (Fig. 57).

Microfilariae found in the lungs in process of dissolution may have undergone macrophage attack locally or they may have been carried to the lungs after being damaged elsewhere in the host. the latter alternative implies in effect their escape either from those viscera that effect the tide's fall, their passage being wholly by the blood stream or from the lymph nodes (that modify the tide's rise and, presumably take a hand in modifying its fall) in which case their passage is by the lymph stream as far as it takes them, and thereafter by the blood stream.

But that this destruction may also take place locally is a conclusion justified by lung sections from Vul. which show an aggregation of cells of the macrophage system limited by a fibrous sheet (Fig. 58), and circulating microfilariae carried into such an aggregation would have entered an area of potential destruction. The frequency in this infection of these pulmonary aggregations may not be estimated from this material. firstly these sections of mine are clearly selected ones, secondly Vul. had schistosomiasis. Now SHAW and GARIEB (1938) reported in Egypt pulmonary schistosomiasis, haematobial or mansonian

in a third of 282 necropsies, it being the cause of death in 2 per cent, Mul died in Porto Rico, where *S. mansoni* occurs and its eggs were present in one of my four sections of her liver. Egg distribution is then patchy even in the liver, so it may not be assumed that, because I have found no eggs in twenty-four lung sections, these organs were free from them. Only wide examinations can settle whether pure bancroftian infection can cause pulmonary macrophage reactions. Nevertheless, already-dead microfilariae have been found in the lungs of the only two infected persons whose pulmonary material has come to me, and cells capable of causing that destruction are massed in certain sections of one of them.

Degenerate microfilariae found in the lungs may then have suffered their mortal injury in them, or before reaching and being detained in them. But it seems that they need not be detained even there, for O'CONNOR (1923, page 37) reported them in blood got from the systemic circulation. The whole matter is judged to be independent of blood tides, it calls for further study and, as now appears, has done so since BAHR wrote (1912, page 119), "*N B* Signs of acute bronchitis were almost invariably noted in these cases (filarial fever disappearance of microfilariae) during the height of fever."

## SECTION V

### THERE IS NO TIDE

#### 1 ESTABLISHED NATURAL TIDES FAIL

Reasoning and controlled observation have led to the conclusion that in the periodic type of infection, the living female worms in a host undertake their parturitions at about the same hour, that the young are carried by the lymph stream to hazards in the lymph tract and by the pulmonary artery to hazards in the lungs, if enough of the young get past these hazards, they cause the rise of the microfilarial blood tide and are then destroyed, mainly in the visceral part of the macrophage system, the observed extent of their destruction being judged sufficient to explain the tide's fall.

Now MANSON reported (1883, page 49, Chart 1) that while he was making 4-hourly microfilarial counts in roughly equal quantities of blood his patient developed orchitis with fever, that thereupon the tidal rhythm was greatly disturbed for a time, and that tidal peak settled to a persistently lower level.

Another instance, that could be more completely studied because death and necropsy followed, was that of Lan, whose clinical notes and pathological material KNOTT sent to O'CONNOR. Lan had microfilariae in night blood when taken to hospital, with acute frontal sinusitis, at St Croix, Virgin Islands. His temperature reached 104° F and microfilariae ceased to appear there. Ninety minutes after his death KNOTT got and fixed axillary and inguinal lymph nodes, testis and spermatic cord. I have some of O'CONNOR's sections of these. The axillary nodes show dead worms already enshrouded in hyaline



"onions"—they had been dead long enough for the host reaction to be so far advanced. In the cord sections are dilated lymphatic vessels, and within them are male and female worms with the following signs of more recent death. They are crumpled and their staining is diffuse both as to the tissues of the adult worms and the contents of the mother worm genital tubes. In one transverse section the worm vagina contains few indistinctly staining microfilariae. In contrast, the surrounding host tissues stain sharply and normally showing that the parasites had died and begun to degenerate during the host's life, their lack of encasement pointing to recent death, presumably at the time of high fever—the date from which microfilariae were no longer found in the blood. Neither O'Connor nor I found microfilariae in the tissues.

In this man microfilariae disappeared from the circulating blood, so there was no tide. All of the worms in the material examined were already dead when it was fixed and since it was got and fixed 90 minutes after the man died the conclusion follows that they died while he lived—the worm changes are marked and the host's tissues normal. Were the rise of the microfilarial blood tide due to worm perturbations, and had all the man's parasites died as the result of his severe illness, the effect would have been just this.

## 2. THERE IS NO NATURAL TIDE.

Evidence has been given above that in the periodic infection, which prevails over most of the earth's surface, the rise of the microfilarial blood tide is due to the fact that worm perturbations are expulsive and synchronized, and its fall to the destruction of the larvae by the host's macrophage tissues. But there is a great area in the South Pacific east of longitude 170° E. where there is not this periodicity where microfilariae may be found circulating in the blood in some numbers at all hours. In theory this lack of periodicity might be due either to lack of synchronization of microfilarial births, or to some unexplained and general blockage of the macrophage system causing delay in getting rid of the young or to both. Evidence is insufficient to clear up this point, for the reports of special expeditions to this area do not state whether the uteri of the different killed worms taken from the same host at the same time were or were not at the same stage of pregnancy or parturition, as they have been in all O'Connor's material from periodic infections. Collection, sectioning and study of non-periodic material on his scale will settle the point.

In the meantime there are these analogies between happenings in periodic and non-periodic types of infection. BATA (1912, Appendix XX) reported of the non-periodic type that microfilariae disappeared permanently from the blood of seven out of nine patients when febrile inflammatory attacks came on as the result of orchitis and lymphadenitis; O'Connor recorded their temporary disappearance during prolonged attack of enteric fever with the temperature running to 104° F.

Infection with *Dirofilaria immitis* shed light here. The adults of this parasite have as optimum habitat the cavities of the right heart of the dog and the microfilariae circulate in the blood into which they have been born. Under foul-smelling treatment WAGGAT and

UNDERWOOD (1934) found that the young ceased to be found in the blood and that the mother worms were either sterilized, or were killed and carried off as emboli into the lungs. KHAW and CHEU (1936) found that this sterilization might be temporary, microfilariae again circulating after an interval, an experience that WRIGHT and UNDERWOOD, too, seem to have had. When, then, the host's macrophage cells have disposed of circulating microfilariae, the death or sterilization of mother worms will bring it about that no more of their young get into the circulating blood.

Now, death of *W. bancrofti* is common, for in O'CONNOR's material from twenty-four persons there were "already dead" worms in that from twenty-two, and worms that had been killed by the fixing in that from twelve, so in the absence of reinfection the peak level of the microfilarial blood tide will drop as each mother worm dies. BAHR's experience of the permanent disappearance of *Mf. bancrofti* from the blood after local inflammations would fall in with the *Dirofilaria* experiment if local inflammation had killed all their mothers, and O'CONNOR's experience of their temporary disappearance is explicable as a temporary sterilizing of all mother worms by the temperature or toxins of enteric fever.

### 3 SECTIONAL SUMMARIZING COMMENT

I suspect that on this matter of periodicity, thought and reasoning have been unduly led by historical sequence. MANSON's ridiculed announcement that in this infection microfilariae make a nightly appearance in the blood, and the later realization that after all he was right, seem to be behind a tendency to explain the yet later discovered natural lack of periodicity as something that has evolved from periodicity. But further consideration strengthens the probability (LANE, 1929) that the simpler non-periodic state is the primitive one. Thus the carrier in the Pacific non-periodic area, *Aedes pseudoscutellaris*, is far more easily and heavily infected than is *Culex fatigans*, the common vector in periodic areas. When parasitism came into being as a necessary stage in the growth of the larval worm, it could hardly have done so except in an area where there lived an animal that was specially susceptible and hospitable to the larva, and that *A. pseudoscutellaris* is. But man, where this mosquito flourishes, was at one time massively migratory and these travellers wandered into areas where *C. fatigans* replaced *A. pseudoscutellaris* in commonness. Now BAHR (1912) showed that *C. fatigans* is far less susceptible to infection than is the *Aedes*. Thus, when specimens of the *Culex* took blood meals estimated to contain 850 larvae, the percentage of these that reached the proboscis and became infective for man was 0.12, when the *Aedes* took meals estimated to contain 2,789 microfilariae the percentage that reached the proboscis was 1.44. *A. pseudoscutellaris* was twelve times as hospitable to the larval *W. bancrofti* as was *C. fatigans*.

The position taken, then, is that these migrant travellers carried this worm from places where the indigenous larval host is easy to infect to others where the prevalent insect host is only one-twelfth as susceptible. That should, other things being equal, have made the parasite a minor menace to man in the

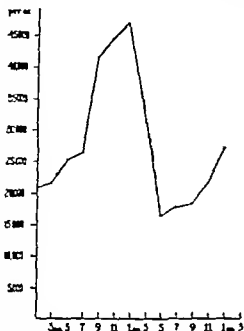


CHART III. Curves selected by H&M as representative of the microfilarial percentage in dog infected with *Dirofilaria immitis*.

from Amer. J. Trop. Med. (1975) 15: 371

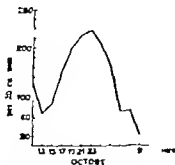


CHART V. A stage count of microfilariae in the blood of the same dogs from Chart IV was obtained, but under next month and in the same conditions.

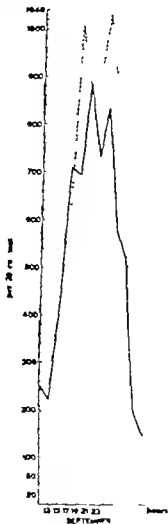


CHART IV. A stage count of microfilariae in the blood of 10 dogs infected with *Dirofilaria immitis*, made by K&M. They are made every 2 hours for 24 hours next fortnight in September. The counts on one dog are shown by continuous line and on the other by dotted line.

from Amer. J. Trop. Med. (1975) Suppl. 375

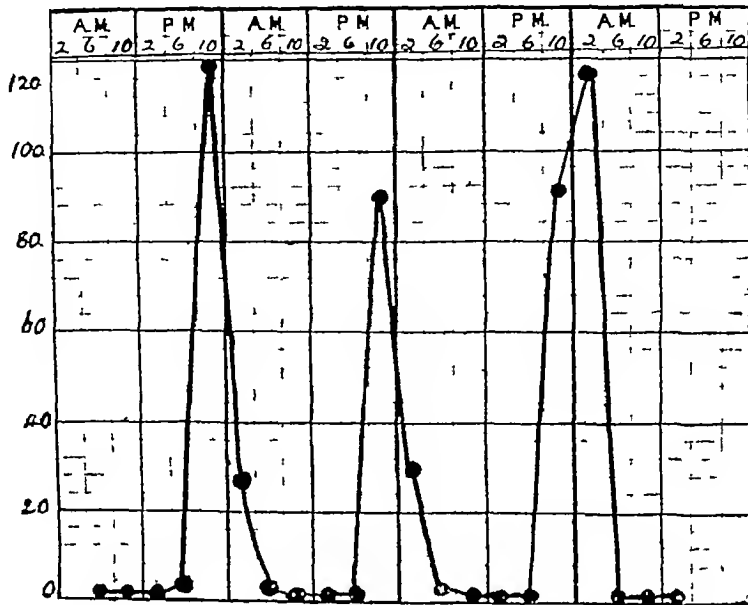


CHART VI "Four hour chart of filaria with nocturnal periodicity"  
The blood was that of an Indian who had been in Fiji for 3 years

From BAHR (1912) *J Lond Sch trop Med* Suppl No 1

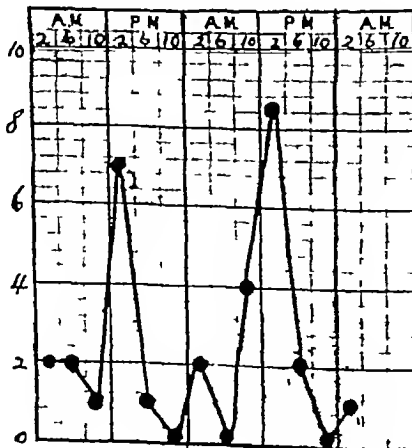


CHART VII. "Four hour chart of filaria with no periodicity"

The blood was that of an Indian who had been in Fiji for 5 years

From BAHR (1912) *J Lond Sch trop Med*



new areas. It has had no such effect. Of this there seem two possible alternative explanations. (a) The fecundity of the mother worms increased when they reached the *Culex* areas, but an increase in the number of young born daily would imply a corresponding increase in the size of the reproductive organs, and no corresponding anatomical differences between the periodic and non-periodic forms or races have, I believe, been put on record. (b) The mother worms so altered their parturitions that the chances of survival of the young in the less hospitable insect were greatly increased. Periodicity would effect this were it of such a kind that the young of the various worms in a host were poured into the blood simultaneously at the hour when it was the new insect host's habit to feed, in other words, were there established a nocturnal blood tide by synchronized worm parturitions, and for the presence of synchronized parturitions evidence has been offered above.

In this line of reasoning a step is missing, the evidence that in the non-periodic, and it is held, primitive race, worm parturitions are not synchronized. No one has thought to go into this matter. Someone some day will perhaps do so, and will make it clear that his data have come from the uterine states of killed worms only.

The suggestion has been made that, although no anatomical differences have been established between the periodic and non-periodic types of this *Wuchereria*, either in the adult or larval stages, nevertheless, by reason of their clinical or biological differences they should be classed as different species, the non-periodic becoming *W. pacifica* Manson-Bahr, 1941. On the evidence set out above, the rise of the microfilarial blood tide results from synchronization of parturitions of the worms parasitizing any host—from simultaneous timing of certain bodily functions. Curious results that may logically follow from acceptance of this basis for the zoological classification of man have been set out (LANE, 1942).

Further pointers are here considered based on variations seen in the microfilarial blood tides when infection is by *Dirofilaria immitis*. Thus HINMAN (1935) set out for this infection "a representative curve of microfilarial periodicity" (Chart III). KUBO (1938) found greatly varying curves on charting average microfilarial numbers in the blood of the same dog at the same hours on consecutive months (Charts IV and V). Finally, BAHR (1912, page 129) had set out two graphs (reproduced here as Charts VI and VII), the former being held to show periodicity, the latter none, a classification that after all would designate *Loa loa* infection as non-periodic. If zoological species were to be separated on biological lines, there would sometimes be needed different zoological names for the same parasite as month follows month.

## SECTION VI

## THE TIDAL MECHANISMS AND DIRECT DIAGNOSIS

Direct diagnosis uses material from a host in order to make visible a parasite at some stage of its life history—usually in this infection the stage is the microfilaria and the material the blood. The aim of diagnosis by blood examination may be qualitative or quantitative.

## 1. QUALITATIVE DIAGNOSIS.

## (1) BY BLOOD EXAMINATION FOR MICROFILARIAE.

A positive blood examination establishes infection—a negative one does not certify its absence as has been known since MANNON (1881), evidently in an early infection, found microfilariae in lymph but none in blood.

KOTT (1936) gave exactness to this knowledge—for by taking tidal mechanisms into account and by using large amounts of laked blood at "low tide" he was able to make

fairly satisfactory direct diagnosis whatever the hour. Venous blood was laked with ten times its volume of 2 per cent. formalin solution, left to stand for 1 to 24 hours, the supernatant fluid poured off and the sediment spread over 8 sq. cm. and stained. He got these percentages: infections in 155 lads at 8 Cross, Virgin Islands, in 20 c.c.m. of blood at 09.00 hours 31, at 1.00 hours 54, in 1 c.c. of blood at 09.00 hours 57, at 21.00 hours 62, in 10 c.c. of blood at 21.00 hours 60. With infections of different general weights the figures would vary in different places.

## (2) BY EXCISION AND SECTION OF TISSUE FROM SUSPECTED SITES OF ADULTS.

## (a) Sites indicated by focal spots.

A focal spot is one from which lymphangitic attacks are prone to start; it may remain tender between these attacks. O'CONNOR, GOLDBER and ADENICKSON (1930) have shown by its excision that beneath it one may expect to find in the subcutaneous tissue a structure of killed and of already dead worms.

## (b) Sites indicated by enlarged lymph nodes.

These are favourite habitats especially about the openings of different lymph vessels into marginal sinus. Every such node in O'CONNOR's material from established infection shows adult worms.

## (c) Sites indicated by X-ray shadows.

These shadows thrown by dead worms, calcified in part or in whole, may well indicate that here, as in other sites, killed worms will probably be mixed with the dead ones.

When these sites are removed in the interest of the host it should, by trying the hour be possible to get increased evidence of the biological mechanisms underlying periodicity.

## 2. QUANTITATIVE DIAGNOSIS.

From blood counts of microfilaria an estimate has been made of the load of mother worms in that host. To be of any value such an estimate must have had as its basis a blood count of microfilariae in some particular host and a count of the mother worms in that same host. Here are some difficulties met with in acquiring that knowledge.

## (i) COUNTS OF MICROFILARIAE IN SYSTEMIC BLOOD

Counting microfilariae in a given quantity of blood got as usual by skin-prick does not establish this basis. BAHR (1912, Appendix II) counted spreads, each of 16 c mm, of such blood from a man with non-periodic infection, made at the same hour of the same day. In the instances where twelve such spreads were made at the same time, the numbers of microfilariae varied between 28 and 67, 42 and 120, 0 and 7, 28 and 69, 65 and 103. YORKE and BLACKLOCK (1917) showed that blood got as usual by skin-prick does not measure the microfilarial content of whole blood, for not only do capillaries magnify this figure but do so, they suggested, to a different extent at different hours of the day. KNOTT's findings (1939) pointed also to this magnification by capillaries, thus at 09 00 hours he collected from each of the same lads 0.02 c.mm. of blood by finger-prick and 1 c.c. by vein puncture, in twenty of them there were microfilariae in the smaller quantity of blood so that the two means of getting the sample were comparable. Vein puncture gave an average number of 61 microfilariae per c mm. of blood, while in finger-prick blood the average was 135, a magnification of more than double.

## (ii) COUNTS OF THE MOTHER WORMS

In my material from O'CONNOR, worms lie in these habitats: (a) lymph nodes in the popliteal space, in Hunter's canal, in sublingual, inguinal and iliac regions, along the aorta to diaphragm level, in bronchial nodes, (b) in subcutaneous tissue, (c) in viscera, namely lungs, adrenals and testicles. MANSON's first report of the finding of an adult worm (1881, 1883) was from scrotal lymphatics. More of such wide collections will probably add to this list, and it may confidently be stated that never yet has the worm load of any host been unquestionably established.

## (iii) OTHER ELEMENTS OF CONFUSION

There are other hindrances to the making of a quantitative diagnosis. It is unknown whether at her nightly parturition each mother worm always empties her uterine tubes down to the same level, so that there cannot be made an estimate based on comparison of the volume of a larva outstretched or coiled and that of emptied sectors of the uterus. It is in any instance unknown what proportion of a brood is destroyed in lymph nodes and in lungs before microfilariae reach the systemic circulation. It is unknown how quickly after reaching this the microfilariae in any host are destroyed in lymph nodes or viscera. Again, accuracy of any such estimation would depend on using the numbers of larvae in the blood at some selected point on the wave chart, the only point that could be fixed is the short-lasting peak, and I recall no instance in which, with the aim of making a quantitative diagnosis, examination has been made at the established peak hour and not at one that should be near it. Estimation of the worm load cannot at present be made successfully by blood examination.

## 3 SECTIONAL SUMMARY

The ordinary blood examination, if negative, does not exclude infection. A negative result in an infected person may be made to give place to a positive one by attention to enriching methods of examination and to certain details that take into account the microfilarial blood tides. Focal spots and the result of X-ray exposures may indicate sites of excisions likely to be useful in adding to knowledge of the tidal mechanism, when the primary duty to a patient allows or demands this.

There is no hope in present knowledge of making a quantitative diagnosis—of estimating the worm load by making microfilarial blood counts. The very bases on which such an estimate must rest are lacking.



## SECTION VII

MECHANISMS OF HOST AND PARASITE IN THEIR BEARING  
ON ANTHELMINTIC TREATMENT

By the evidence set out, the rise of the microfilarial blood tide is due to synchronized nightly parturations by mother worms, and in the main it is the young so born that cause the ill-effects of this infection whether it is or is not periodic. A chief aim in treatment must then be to put a stop to parturations with safety to the host and by methods most suited to effect that end in any particular host.

## 1. SURGICAL EXCISIONS OF WORMS.

Surgical removal of all mother worms would effectively preclude further birth of young.

KNOTT's necropsy on *Mud.* was the most thorough examination in my knowledge and it disclosed worms in every one of the various sorts of habitat known for her sex, almost all lymph nodes below the clavicles including the bronchial (the popliteal and one in Hunter's canal being found infected in someone else) and certain viscera (to which the testicle and spermatic cord have to be added). It will perhaps not be doubted that equally minute examinations will add to the list. A second significant report is that by O'CONNOR, GOURLEY and AUCHINCLOSS (1930) who on diagnostic X-ray exposures of one lower limb found fifteen shadows or groups of shadows such as are thrown by calcified worms and they would all have to be excised in an attempted surgical unworming because of the usual admixture of living and dead worms in any one site. A third significant report is that by O'CONNOR and HULKE (1933 Case 2) on Ric. she had about 1 000 microfilariae in 20 c.c.m. of night blood, and enlarged and hard epitrochlear and groin nodes. To lessen her infection it was thought advisable to remove these, but the microfilarial night count thereafter varied between 600 and 1 600 and from what has been set out above they were, presumably new births. To be satisfied that excision of material containing living worms has removed all living worms from a host is at present impossible yet that very assumption has been so confidently made that, when microfilariae have persisted in night blood, the incident has been held as clear proof that in the body they are long lived, hide somewhere by day but emerge at night and cause the tidal rise. *Non agellus*.

The Auchincloss operation lightens a heavy limb by removing elephantoid tissue. It is unlikely to unworm completely and does not insure against local recurrences of elephantiasis. Amputation of an elephantoid limb has indeed been followed by elephantiasis in its pair. After excision of nodes Ric. experienced her first lymphangitis with any surgical operation should go preventive anti-bacterial treatment—vaccine serum, or such drugs as protozol or sofram.

## 2. DRUG TREATMENT

The life of *W. bancrofti* and the prospect of ending it by drugs are bound up with man's two internal transportation circuits (1) the blood circuit within the blood vessels (2) the plasma-lymph circuit by which plasma and red cells that have seeped from the first circuit through the capillary walls pass under the names of tissue fluid and lymph along the lymphatic vessels,

These measures are equally needed in treatment of lymphangitic attack.

to rejoin the blood circuit through the thoracic and right lymphatic ducts. The habitat of the adult parasites is in the lymph circuit, but an infective larva entering man from mosquito may find itself in either circuit, and in view of what will be its adult site it presumably becomes restless when not in lymph, thus boring urge persisting till it reaches lymph. Such an instinct would explain the way in which it settles down in lymph nodes and in lymphatics such as those of the scrotum. It is these spots which drugs have to reach.

There are three essentials for successful drug treatment (a) it must be no more dangerous to the host than is the infection itself, it may not get rid of a focus of infection or reinfection at greater risk to the host's life or health than this causes (b) It must kill, or at least permanently sterilize, the female parasites, so that the host is no longer a risk to himself or others (c) Its route of administration must be one that will take the drug to the parasites in the concentration that will best effect these ends. It is here suggested that to none of these essentials has sufficient attention been paid.

#### (i) SAFETY OF DRUGS

The worm rarely kills directly and a quotation from Queen Anne's celebrated physician illustrates this and the next requisite. About 200 years ago he advised as anthelmintics against intestinal worms —

"All things that are known to kill them as oils of all kinds, Honey taken upon an empty Stomach or after some gentle purging Medicine, Substances which by their small pungent and sharp particles kill them without hurting the Intestines as all Fish-Bones and Hartshorn powdered" (ARBUTHNOT, 1735)

It is easy to smile at JOHN ARBUTHNOT,\* perhaps wiser to consider whether there is not a present tendency to act as if we already had all the knowledge essential to insure safety in anthelmintic drugging. For example, since calcification is common in adult *Wuchereria*, and has been seen in its microfilariae already dead in the host, may they not have been killed by calcification? BRAND, OTTO and ABRAMS (1938) tested this idea on the larvae of *Trichinella spiralis* by giving parathormone hypodermically to laboratory-bred rats. They could not produce calcification of larvae without causing calcification of the host's tissues, widespread enough even to cause its death. Only after their death did the young nematodes calcify, and we may not let anthelmintic enthusiasm kill the helminth's host.

#### (ii) CONTROLLED APPRAISEMENT OF DRUGS

In controlled appraisalment of anthelmintic drugs in this infection, British Empire Schools of Tropical Medicine have taken a notable share. O'CONNOR (1923), then of the London School, reported from the Western Pacific on the action of ten drugs given mostly intravenously, but also subcutaneously and by mouth, and found no subsequent change in numbers, structure, or behaviour of microfilariae. In the expedition under LEIPER sent by the same School to British Guiana, ANDERSON (in ANDERSON, KHALIL, LEE and LEIPER, 1923), detected no effects on microfilarial numbers in the blood on testing twenty-two substances. CHOPRA and SUNDAR RAO (1939), of the Calcutta School, tested seventy-six substances on 470 persons and reported that "so far no drug has been found that has satisfactory anti-filarial properties," for, though foudrin given intravenously evidently gave temporary sterilization, microfilariae reappeared in the blood.

\* Yet the title page of his book cites him as M.D., and Fellow of the College of Physicians of London and Edinburgh, and of the Royal Society.

few days later this drug cleared up chyluria but caused gastritis and enteritis. HAWKINS (1940) from the Liverpool School, tested eleven substances including fousadin given intravenously, intramuscularly and orally but none lessened microfilarial numbers in the blood. From these investigations and from analogies with *Dirofilaria immitis* we have guidance. *D. immitis* has as optimum habitat the right heart cavities of the dog. Injection of fousadin into the dog's veins has resulted in sterilization, temporary or permanent, of the worm, or in its death and its transport as an embolus into the lungs (WALKER and UNDERWOOD 1934). Sterilization has shown itself during life by temporary or permanent disappearance of microfilariae from the host blood, and it was controlled after the host death by absence of young from the uterus of mother worms. Now the temporary disappearance of *M. bancrofti* from the blood in CHOPRA and RAO's experience was by inference caused, as was that in WALKER and UNDERWOOD's work, by temporary sterilization of mother worms. May not this drug-induced infestation or death of mother worms be effected in infection by *M. bancrofti* as it has been in that by *D. immitis*? Consideration suggests that it might be done by attention to the route of administration.

#### (m) ROUTE OF DRUG ADMINISTRATION THE LYMPH CIRCUIT

When WALKER and UNDERWOOD (1934) injected fousadin into the veins of dog in the cavities of whose right heart there lived *D. immitis*† they used route which directly and in efficient concentration and quantity carried the drug to the worm. The analogous procedure in bancroftian infection would be its injection into the lymphatic stream at spot where this would carry it to particular suspected habitats. The successful use in this way of fousadin, or of such other drug toxic to the species, will depend on two factors: (1) knowledge of occupied habitats. (2) The entry of the drug into lymph vessel that will carry it to those habitats. I have listed established habitats and noted signs and symptoms which suggest that in particular person particular sites have been occupied by worm. There seem to be ways of so acting that the drug directly enters the lymph of worm habitat: the first is its injection into the place where the worm is suspected to lie, such as an enlarged lymph node, particularly one that is varicose; the second is to find and put it into lymphatic vessels whose current runs to the suspected habitats. I do not think that the use of either method has been reported and have no suggestion to make regarding the first.

On the second possible line of action hopeful guidance is given by the technique worked out by DICKER and his colleagues to make these vessels visible in the leg of the dog. A soluble dye is injected between the toes and is, by gentle massage made to pass on into lymphatic trunks and then to make visible those under the epidermis, into one of which hollow needle is introduced and drug run through it. The substances that were so run into it in DORTON were used to cause elephantiasis by obstructing the flow through it. It seems reasonable to hope that drug, perhaps fousadin itself, may be found that will prevent or outpace certain elephantiasis by killing or sterilizing the mother worms that are the primary cause of its appearance in bancroftian filariasis. But this possibility of intra-lymphatic drug-giving has another problem. When elephantiasis has been produced in dog, the injection of soluble dye is followed by its drifting about in the oedematous fluid, whence it does not pass on into lymphatic vessels. Yet these vessels do persist in the elephantotic limb, so it needs determining whether after removal of the oedematous fluid, as by bandage boot (HUNTER 1935) or some other pressure and gravity method, dye injection may not be made to flow along lymph vessels, display them and allow even at this late stage their use for sterilizing or destroying mother worms instead of, as hitherto, for nourishing them.

More recently in America, CULBERTSON and others (1941 and 1947) have had greater success in treating infections with *Leishmanoides caryae* in the cotton rat, and human infections with *Wuchereria bancrofti* using organic anticonvulsants such as bromobutenol, though none could be considered to be ideal.

† They were the first to do so, including HARRIS and CURRY (1934)

### 3 X-RAY TREATMENT

The conclusion that X-ray irradiation may interfere with the tidal mechanism of this infection is still inferential for it does not seem to have been checked.

GOLDEN and O'CONNOR (1934) reported that from two patients, Bry and Deb, lymph nodes were removed 2, 3 and 8 months after irradiation and that their condition was exceptional in that all the worms in the material were already dead (there was not the usual mixture of these with worms killed by the firing). In each case a group of groin nodes had been treated, in Bry, fifty-one worms, in Deb, eighty worms were identified in the serial sections. Calcification of these worms was often limited to their body surface, the collection of round cells about them was held to be larger than usual, and among them there were more giant cells, these conditions suggest recent death.

With this should be considered the report of LEVIN and EVANS (1942) that irradiation of *Trichinella spiralis* in the rat has been so carried out as to sterilize these worms.

X-rays are then capable of sterilizing and, it seems, of killing nematode parasites—of doing just what is needed for dealing with *W. bancrofti*, and in its periodic form for suppressing the tidal mechanism. Convincing evidence on the matter must be accompanied by microfilarial blood counts on irradiated patients, but the meagre tentative evidence is suggestive and hopeful in sites where the rays may be used without risk to the host. Here an experience of GOLDEN and O'CONNOR (1934) is apposite, they used with benefit the rays to relieve chyluria, applying them over the receptaculum chyli (this mechanism of action merits investigation) and produced in their patient a temporary amenorrhoea, evidence that the rays may cause a temporary sterilization in both host and parasite.

### 4 TREATMENT BY HEIGHTENING IMMUNITY

When considering the larvicidal mechanism that interferes with the rise of the microfilarial blood tide (page 751), it was noted that the production of hyperimmunity to *Nippostrongylus muris* lessened the host's local reaction to the parasite's larvae. Since as noted above, the ill-effects of infection with *W. bancrofti* are mainly attributable to the host's local reaction to its larvae the possibility of bringing about a deliberately-induced hyperimmunity to this parasite, as a means of lessening those ill-effects, brings hope of bettering treatment.

### 5 TREATMENT BY LYMPH THROMBOSIS

The possibility of inducing lymph thrombosis and thereby of keeping microfilariae out of the blood holds little prospect of success. Its effect must be temporary, owing to the readiness with which lymph flow is re-established in a clot-filled lymphatic, and the procedure is not hopeful owing to the wide range of known worm habitats.

### 6 SECTIONAL SUMMARY

Surgical excision of worms holds little or no prospect of unworming the usual patient, but if for any reason excision is advisable, anti-bacterial treatment should go with it. British Schools of Tropical Medicine have been pre-eminent in appraising anthelmintic drugs in this infection, the drugs have not been given by the lymphatic route, though this seems the most promising. Controlled X-ray irradiation has given some hopeful results. Solution of a worm's body wall and of one uterine branch while within the host's body have been seen. Is it too much to hope to effect this deliberately? Treatment by inducing hyperimmunity and so lessening the harm the parasitic larvae do to the host is worth considering. The prospect for betterment of treatment looks promising.

## SECTION VIII

## CONCLUSIONS.

The sectional summarizing comments may be summed up in these conclusions based on detailed examinations of some 800 microscope slides given or bequeathed to me by the late Prof. F. W. O. COXSON. In the periodic type of infection the nightly rise in the microfilarial blood tide is due primarily to synchronized parturations by female worms, which probably precede the rise of the tide by a few minutes. Provided they are not overtaxed, this rise is modified or annulled by the active cells of the host's macrophage system recruited in the lymph tract and lungs. This repressive reaction, called into being by adult, and especially by larval, worms results in an excess of lymph protein, which excess sets going in lumbar, scrotum and mammae the changes that give rise to hydrocele or elephantiasis. The fall of the microfilarial blood tide is due to the destruction of the nightly brood in liver, spleen and adrenals, probably in lymph nodes and possibly elsewhere—the normally high (and locally mucous) protein content of visceral lymph is not likely thereby to be significantly and harmfully raised.

In making a diagnosis microfilarial counts of skin blood cannot estimate with useful accuracy the worm load, but detection of microfilariae in this can be made more certain by enriching methods, and by getting the blood at the usual peak hour of the microfilarial blood tide.

The absence of tide is explicable either by lack of synchronization of worm parturations, or by blockage of the host's macrophage system.

The acute inflammations that are features of this infection have been traced to superadded bacterial infection and are amenable to anti bacterial treatment. This should be given prophylactically where surgery is needed.

There is little hope of complete unworming by surgical excisions—for the worm habitats in O. COXSON'S material and experience comprise lymph nodes from Hunter's canal to the retrothoracic region and bronchial and axillary nodes, the subcutaneous tissue, the lungs, the adrenals and the testicles—and there may be yet other habitats. But surgery will right inconvenience or will remove a focal spot.

In non-surgical treatment the use of the blood to convey a drug in adequate concentration to worm habitats has little prospect of success; that of the lymph stream to selected habitats seems more promising, provided a specifically toxic drug is found. X-rays have sterilized and killed worms and should be able to cut out the rise of the blood tide and, with that, the host's self-damaging reactions to newborn microfilariae, provided they reach all mother worms. As always the risk to the host by treatment must

be less than that resulting from the infection itself. Over parts of the earth's surface there is no microfilarial blood tide, the causative mechanisms being ineffective or absent. Equally the tides of a periodic infection may fail, sterilization or death of mother worms interfering with their rise, or blockage of the macrophage system interrupting their fall.

At present the position in this infection falls in strangely with words used by TOPLEY (1940) in his Linacre lecture —

"Science has tried and condemned authority, and in its grosser forms authority is dead. Where it still survives in the field of medicine it might, I think, be defined as didactic assertion of inadequately tested working hypotheses, accompanied by an unwillingness to meet the challenge of a critic by performing a more adequate series of tests."

Investigations need not be on O'CONNOR's magnificent scale in order to determine whether in non-periodic regions worm parturitions are or are not synchronized, as they have been in all his material from areas where the infection is periodic.

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# THE PRE-ERYTHROCYTIC DEVELOPMENT OF PLASMODIUM CYNOMOLGI AND PLASMODIUM VIVAX

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The discovery 12 years ago of an additional phase in the developmental cycle of *Plasmodium*, gave a great impetus to malaria research. The exo-erythrocytic cycle in avian parasites was quickly elucidated (e.g., JAMES and TATE, 1938, RAFFAELE, 1936), but in spite of careful work in many parts of the world, the analogous stage in mammalian malaria remained unknown until recently. The earlier work is reported in an extensive literature which it is not proposed to discuss here, useful summaries have been made by HUFF and COULSTON (1944), who dealt mainly with avian plasmodia, by DAVEY (1946), who emphasized the chemotherapeutic aspect, and by ANGELINI (1947), who considered the various doubtful records in human malaria.

It became increasingly clear from these investigations that SCHAUDINN'S (1903) assertion regarding the direct entry of sporozoites into erythrocytes did not fit in with the new knowledge. In particular, his theory could not be reconciled with two well recognized phenomena. Firstly, there was the remarkable difference in the course of blood-induced as compared with sporozoite-induced infections. In the former there is no real incubation period and parasites rapidly become demonstrable in the peripheral blood, after sporozoite inoculation, on the other hand, there is a clear-cut incubation period varying in length according to the species of parasite, throughout which the blood is non-infective on sub-inoculation. The second discrepancy is in regard to the response of these two types of infection to anti-malarial drugs. After blood inoculation the infection is promptly eradicated by the drug, whereas after

sporozoite inoculation, the disease will make its appearance, sooner or later in spite of the drug.

Researches on avian malaria sufficed to explain these anomalies, so far as avian malaria was concerned, by demonstrating the existence and the nature of the tissue phase, and it became so obvious that a similar phase must exist in the human species that such a cycle was even prematurely included in some descriptions of the life history of the malarial parasites of man.

Early this year our own experiments with *Plasmodium cynomolgi*—a malarial parasite of oriental monkeys—met with success and brief descriptions of the pre-erythrocytic cycle in the liver were given (SHORTT and GARNHAM 1948a, and SHORTT, GARNHAM and MALAMOS, 1948). We had no doubt that the very closely allied *P. fivax* of man would exhibit similar forms, and shortly afterwards we were able to demonstrate these in the liver of a human volunteer (SHORTT, GARNHAM, COVELL and SHUTZ, 1949).

The present paper gives a detailed account of the pre-erythrocytic parasites of human and monkey infections. In the former case the description applies to forms present on the 6th and 7th days of the incubation period while, in the latter stages seen on consecutive days from the 5th to the 10th days are described.

#### MATERIALS AND METHODS.

The materials necessary in our experiments were anopheline mosquitoes, *Anopheles maculipennis atroparvus* monkeys, chiefly *Macaca mulatta*, but *Cercopithecus aethiops* was also used a human volunteer and strains of *P. cynomolgi* and *P. fivax*.

The strain of *P. cynomolgi* came originally from the Central Research Institute of India, Kasauli but was given us in 1945 by Dr F. HAWKING, of the Medical Research Council who had obtained it from Dr F. WOLFSON of the School of Public Health, Johns Hopkins Medical School, Baltimore. The strain of *P. fivax* was a Madagascar strain in use for therapeutic purposes at the Horton Hospital for Mental Diseases, Epsom.

The mosquitoes were bred in our laboratory but the numbers were reinforced from time to time, when necessary from other sources.

The monkeys were obtained either through the Medical Research Council London, or by purchase.

The general procedure followed in the individual experiments was first to select an infected animal showing numerous gametocytes in the peripheral blood. On the animal as many anophelines as were available were fed and, whenever possible the same mosquitoes were re-fed once or even twice on the same or another gametocyte-carrying animal. This formed the infecting stock for the experiment and the mosquitoes were thereafter maintained on raisins, occasionally substituted by cube sugar and kept at temperature ranging between 25 and 27°C. and relative humidity of over 80 per cent.

They were next fed on the animal it was hoped to infect and after feeding, the whole mosquitoes were ground in a mortar in heparinized monkey plasma diluted with an equal volume of normal saline solution. The mosquito suspension was then inoculated intraperitoneally or intramuscularly or in both ways, into the same animal.

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The feeding of mosquitoes on the monkeys was carried out by anaesthetizing the animal with nembutal and putting it bodily into the cage of mosquitoes. The effects of the anaesthetic lasted for 1 to 2 hours and the mosquitoes could feed without interruption for the whole of this period. If the mosquitoes had previously been kept without other food for 24 to 48 hours, the great majority would feed at once under the conditions described.

The animals so infected were examined in two ways. Either a special day during the incubation period was selected and the monkey was killed for postmortem examination, or a biopsy was performed on the liver. This could be repeated frequently on the monkey so that the infection could be followed up in the same animal from the early stages until the establishment of the blood infection or even later. The biopsy is a simple operation, the haemorrhage from the cut surface of the liver being easily controlled by cautery.

## FIXING AND STAINING TECHNIQUE

Many fixatives (Zenker, Formol saline, Flemming, etc.) and stains were tried by us in order to obtain the best picture of the parasites in tissue sections, but one method gave results so incomparably better than the others that we adopted it as a routine. The method, in brief, was to fix tissues in Carnoy's fluid—the formula with chloroform—and to stain with Giemsa stain by the method described by SHORTT and COOPER (1948). All the descriptions given below refer to specimens prepared by this method.

Before proceeding to a description of the parasite, we give below an account of the actual experimental details of one monkey and of the only human experiment.

## EXPERIMENTS

MONKEY 27 *Macaca mulatta*

About 1,000 mosquitoes were infected with *P. cynomolgi* by three successive feeds on gametocyte-carrying monkeys. An interval of 10 days was allowed to elapse after the third feed. During this period the mosquitoes were fed on raisins and cube sugar and were kept at a temperature ranging between 25° and 27° C and a relative humidity of 80 per cent or over.

A sample of twenty mosquitoes was dissected and all proved heavily infected. The survivors numbered 576, and these were allowed to feed on Monkey 27, over 500 did so. The entire batch of mosquitoes was then ground up in a mortar in heparinized monkey plasma diluted with normal saline solution. Half the resulting suspension was inoculated intraperitoneally into the same monkey and the remaining half, in two portions, into the thigh muscles. The monkey was sacrificed 7 days later and material for examination removed from the following tissues—

Spleen, liver, kidney, suprarenal gland, pancreas, small intestine, lymph glands, peritoneum, lungs, heart, thoracic glands, bone marrow, brain, leg muscles, stretch preparations of pia mater and omentum.

All the tissues obtained are under examination but, so far, tissue stages of the parasites have only been found in the liver.

## HUMAN SUBJECT (A C)

The general procedure was the same, with the following differences. 3,600 mosquitoes were infected with *P. vivax* by two successive feeds on a gametocyte-carrying malaria case. The mosquitoes were kept at a temperature ranging from 24°–26° C and a relative humidity of 75–80 per cent and were fed on rabbits. Fourteen days after the last infecting feed, the survivors were allowed to feed on A C on 2 successive days. A total of 2,010 fed, and sample dissections showed 86 per cent of these to be infected. In addition, the salivary glands of 200 of the mosquitoes were dissected out in Locke's fluid and inoculated intravenously into the subject. Seven days later a biopsy of the liver was performed.

## DESCRIPTION OF PRE ERYTHROCYTIC FORMS.

(a) *Plasmodium cynomolgi*

The earliest forms seen were those occurring on the 5th day of the incubation period and the succeeding stages, day by day up to the 10th day have been found and examined. These will be described in detail below.

**Fifth Day Stage (Fig. 1).**—The parasite, which is found in the parenchyma cells, is a spherical or ovoid body measuring about  $10.5\mu$  in the longest diameter. The cytoplasm is granular and stains a blue or mauve-blue. The chromatin is in irregularly-shaped masses staining a purple or magenta colour. The disposition of the chromatin in many portions indicates that the body represents an early stage in schizogony of the parasite, the fragments of chromatin numbering about fifty in an entire schizont followed in serial sections. The outline of the schizont is very clear-cut and stands out in sharp contrast with the cytoplasm of the liver cell. The nucleus of the liver cell is eccentric owing to the presence of the parasite but, as a rule, is not deformed at this stage, and the staining reaction of cytoplasm and nucleus is unaltered.

**Sixth Day Stage (Fig. 2).**—The forms found indicate that there is very rapid growth at this stage. The schizont is ovoid and measures  $18\mu$  in the longest diameter. The cytoplasm is granular and stains a pastel shade of blue. The masses of chromatin stain a magenta colour and are now much more numerous, numbering not less than 100 in an entire schizont and scattered fairly evenly throughout its substance. The nucleus of the host cell is displaced to one side but may still be little changed in shape and there is no difference in staining reaction. The smooth contour of the parasite at all stages and whatever its shape would appear to indicate some kind of containing pellicle but this, if present is too fine to be apparent. In sections the parasite may sometimes be shrunken and there is then a clear space between it and the cytoplasm of the containing cell. This space may completely surround the parasite or appear opposite part of the circumference only.

**Seventh Day Stage (Figs. 3-6).**—The parasite has again increased in size

## PLATE I

SCHIZONT OF *PLASMODIUM CYNOMOLGI* DURING THE INCUBATION PERIOD

FIG. 1.—On 5th day

FIG. 2.—On 6th day

FIG. 3.—On 7th day showing entire form without vacuoles.

FIG. 4.—On 7th day showing form with lateral vacuole.

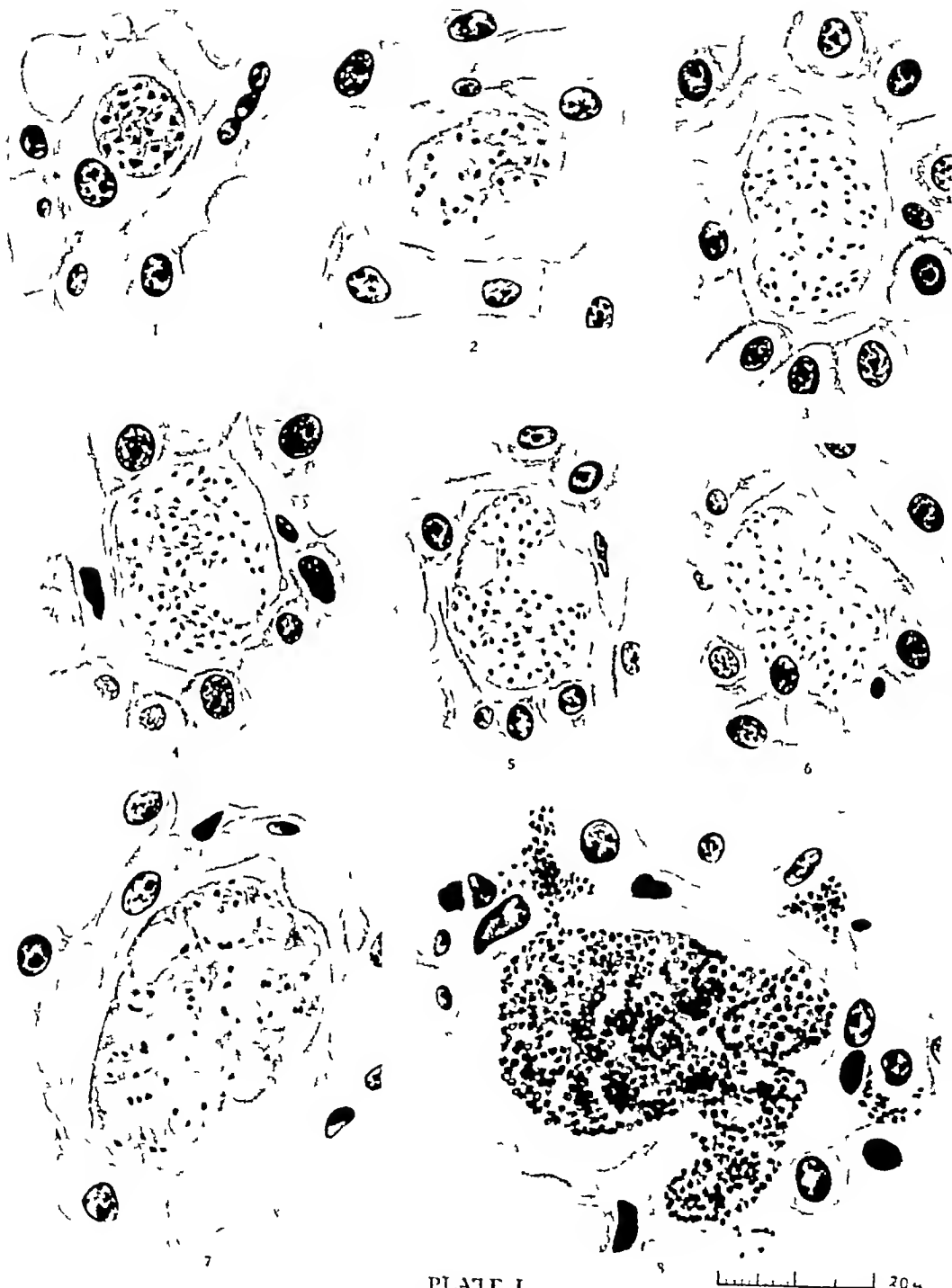
FIG. 5.—On 7th day showing form with lateral indentation.

FIG. 6.—On 7th day showing form with lobose arms.

SCHIZONT OF *PLASMODIUM ILLAX* DURING THE INCUBATION PERIOD (page 791)

FIG. 7.—On 7th day showing form with vacuoles.

FIG. 8.—On 7th day showing more advanced stage with release of merozoites.



# PLATE I

FIGS 1 to 6 —Schizont of *Plasmodium cynomolgi*  
 FIGS 7 and 8 —Schizont of *Plasmodium cynomolgi*







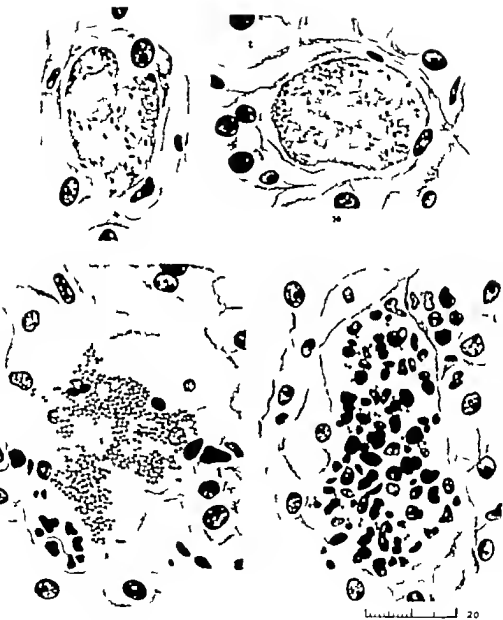


PLATE II  
 1 to 12 Schizont of *Plasmodium cynaoabae*

while retaining its general ovoid shape except where this is altered as described below. It now measures up to  $31\mu$  in its longest diameter. The outline varies considerably, and this is probably due to unequal pressure exerted on different parts of the periphery. Thus, there are regular ovoid forms, forms where the regular curve of the contour is altered by neighbouring resistant structures such as fibrous tissue or larger vessels, and some forms with one or more lobose arms.

In the parasites one or more vacuoles, small or large, are frequently present. When the vacuole occurs near the periphery it has the effect of producing an apparent indentation of the parasite. The lobose form is possibly also produced to some extent in this way. Whether these vacuoles are all true vacuoles, for some certainly appear to be so, or whether the structure may simulate vacuolation by the cutting of folds in the parasite, we are not sure. Whichever is the true explanation, the structure is possibly connected with the respiration of the parasite or the production of a larger absorbing surface.

The cytoplasm of the schizonts is now coarsely granular and stains a pastel shade of blue. Here and there are masses staining a darker blue and looking like local condensations of the cytoplasm. The chromatin fragments stain a magenta colour and are now very numerous and scattered fairly uniformly. An estimation made of their number from a parasite examined in serial sections gave the figure of 800 to 1,000. This nearly mature stage shows a close resemblance to an early stage in the development of *Hepatocystes kochi* in the liver of African monkeys and the latter might be a cause of confusion if they were used as experimental animals.

The 7th-day stage was also studied in impression smears of liver. Here the parasites are seen as irregularly ovate bodies sometimes entire, but more often ruptured due to trauma in making the smear, and in such cases the chromatin particles are found escaping but are not yet in the form of fully mature merozoites.

*Eighth Day Stage* (Fig. 9).—The majority of the schizonts show but little difference from those of the 7th-day in their morphology and size. In some, commencing condensation of the cytoplasm around the chromatin masses, to form merozoites, is indicated but in sections this is masked to an extent which would be less evident in a smear preparation.

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## PLATE II

### SCHIZONT OF *PLASMODIUM CYNOMOLGI* DURING THE INCUBATION PERIOD

- FIG. 9.—On 8th day, showing form with lateral indentation.  
FIG. 10.—On 9th day, showing entire form without vacuoles.  
FIG. 11.—On 10th day, showing form ruptured and releasing merozoites.  
FIG. 12.—Invasion by phagocytes of the area previously occupied by schizont of *P. cynomolgi* on 10th day of the incubation period, active phagocytosis of remaining merozoites in progress.

*Ninth Day Stage* (Fig. 10).—The schizonts show some increase in size merozoite formation is well advanced and in many cases complete. An average size for the schizont is now  $30\mu$  in the longest diameter but forms up to  $43\mu$  may be encountered. The staining reactions of cytoplasm and chromatin are unchanged.

*Tenth Day Stage* (Figs. 11 and 12).—The picture at this stage is completely changed. The formation of merozoites in the schizonts is the rule even where the latter are still entire. Three stages in schizogony are now to be seen and will be described in the sequence in which they occur.

In the first place there is the mature schizont containing merozoites, but still intact within its containing membrane. This is similar to the forms seen on the 9th day.

Following on this stage is the newly ruptured schizont seen as a central mass of closely packed but discrete merozoites with smaller groups and individual merozoites infiltrating the surrounding tissue. These merozoites each consist of a small fragment of chromatin with an attached portion of blue cytoplasm. They are sharply defined little bodies varying in shape and about  $113\mu$  in diameter. A new feature in the cytological picture now appears.

During the growth of the schizonts, from the 5th day onwards, there is no reaction to their presence on the part of the liver. As they increase in size they expand the containing liver cell and compress its nucleus until finally the schizont lies in a space, the walls of which appear to be the attenuated remains of the liver cell with, possibly other compressed material outside this.

A dramatic change is seen when the schizont ruptures and releases its merozoites. There is an immediate invasion of the area by phagocytic cells which proceed to engulf large numbers of the merozoites, but one presumes that most of the latter if not the majority attack the red cells to initiate the invasion of the blood stream while the laggrards are engulfed by the phagocytes and digested. The phagocytic cells at first are few in number and may be seen thinly scattered among the merozoites of a ruptured schizont.

As the invasive process proceeds, there is found an increasing number of the phagocytes and a corresponding diminution of the number of merozoites. The infiltration of cells forms an island in the otherwise uniform liver substance and stands out in marked contrast to the latter. The invading cells are mainly monocytoïd but polymorphonuclears are also present and a few plasma cells are to be seen. At a somewhat later stage, the infiltration alone is evident, the merozoites having completely disappeared either by entering other cells or owing to digestion by the phagocytes. One presumes that eventually the latter also will disappear and the area resume an appearance normal to liver tissue.

(b) *Plasmodium vivax* (Plate I, Figs 7 and 8)

*Sixth and Seventh Day Stages*—As the infection of the human case was effected on two successive days, and the biopsy was performed on the 7th day after the first infecting feed, it follows that forms of the 6th and 7th days would both be present. The distinguishing of these one from another could only be presumptive and based on the degree of development. Two stages in development could be distinguished and these gave us the impression either that development was somewhat precocious in this case or that the human parasite develops somewhat more rapidly than the simian.

The earliest forms were ovoid plasmodial masses similar to those seen in the case of *P. cynomolgi* at the same stage (7th day) but were larger, being about  $42\mu$  in the longest diameter (Fig 7). The cytoplasm and chromatin stained respectively in a similar manner and the varied shape, vacuolation and absence of tissue reactions were the same. An estimate of the number of chromatin masses at this stage in an entire parasite gave the number as about 800. In this group somewhat more advanced forms were included which, while retaining the general characters described, showed indications of the condensation of cytoplasm around the chromatin masses to form merozoites, the individuality of the latter being largely masked as in the case of *P. cynomolgi* while the schizonts were still intact.

The 2nd stage seen consisted of a single form (Fig 8) which appeared to represent the rupture of a fully developed schizont and the escape of merozoites from it. We are not entirely satisfied that the appearances seen merit this interpretation and that the rupture was really the spontaneous breaking up of a fully mature schizont. There remains the possibility that a nearly mature schizont was mechanically ruptured with escape of the merozoites into the immediate neighbourhood. Whatever the correct interpretation, there is no doubt that schizogony was far advanced with the production of a very large number of chromatin masses which were being extruded singly and in groups. These units are so small that it is difficult to be sure if there is a cytoplasmic accompaniment to each fragment of chromatin to form a fully developed merozoite. If so, the merozoites are definitely smaller than those of *P. cynomolgi*, and we consider the more likely interpretation of this structure to be that it is a prematurely ruptured schizont in which the merozoites had not reached maturity.

Before leaving the subject of the human experiment, one very striking phenomenon connected with immunity must be mentioned. The human subject had undergone malarial therapy  $22\frac{1}{2}$  months previously by infection with the homologous strain of *P. vivax* induced by blood inoculation and had experienced thirteen peaks of fever. We were uncertain how this previous infection would affect the experiment, but when examination of the biopsy material revealed the presence of schizonts in the liver on the 7th day, we looked confidently for the onset of clinical malaria and parasitaemia about the 9th or

10th day. In actual fact, there was no clinical attack beyond a transitory slight rise of temperature on the 15th day and no parasites were ever demonstrated in the peripheral blood. The significance of this finding will be dealt with in the discussion to follow.

### DISCUSSION

This work has provided a solution to the problem of pre-erythrocytic schizogony in mammalian malaria: there still remain a number of unsolved questions and some of these are discussed below.

The history of the *P. vivax* experiment presents an interesting study in immunity. The important part played by cells of the reticulo-endothelial system in the immune processes occurring in malaria is now well recognized. Observations in mammalian malaria have, however, been confined until recently to the erythrocytic infection, whilst even in avian malaria, where the existence of exo-erythrocytic schizogony has been known for more than a decade, little work appears to have been done on the specific effect of immunity on the tissue phases.

There is, however, some indirect evidence bearing on the question. BORN (1947) in summarizing his experience with *P. vivax* malaria, comes to the conclusion that whilst a very effective immunity is easily obtained against the trophozoites of a homologous strain, the earlier stages, *i.e.*, the sporozoites or their immediate successors, are inaccessible to the immune mechanism. The effectiveness of immunity following a single attack is found to vary considerably. It is usually complete, but mild infections can occur following re-inoculation with the homologous strain. The immunity may last according to BORN THOMAS and KITCHEN (1936) for 3 years or more. With *P. cynomolgi* on the other hand SHORTT, PANDIT, MENON and SWAMINATH (1938) demonstrated that monkeys cured of the infection were as susceptible as normal monkeys to re-infection with the homologous parasites. We have recently amplified this observation by showing that a monkey naturally cured of a blood-induced infection was fully susceptible to a sporozoite infection.

Our work throws a little more light on the problem. It will be noted in the human case that in spite of the apparent absence of blood infection, the liver contained pre-erythrocytic parasites. In other words, development of the sporozoites proceeded unchecked in the liver parenchyma though once the merozoites attempted to establish themselves in the peripheral blood the immunity mechanism immediately suppressed the blood cycle. There is some evidence that there is a cellular barrier for this process. We have shown that on the 10th day following inoculation of *P. cynomolgi* sporozoites, phagocytosis of rupturing schizonts is evident. In the same tissue we observed foci of infiltration which apparently represented the completed stage of this process. In the human liver this appearance was present to a much greater extent though not in direct association with the parasites (which were, at the most, 7th day

forms) The foci here were very numerous, were often a quarter of a millimetre in diameter and consisted of lymphocytes, endothelial cells, plasma cells and, rarely, polymorphonuclears. They lay usually in relation to branches of the portal vein. VINT (1931) has shown that round-celled infiltration involving the portal vein is a very common feature of livers of human beings living in tropical Africa and much exposed to malarial infection. It is not improbable that all these focal infiltrations may represent the final stage in the attack on pre-erythrocytic forms, in the human case they were perhaps more numerous because the patient was in a state of active immunity which even acted to some extent on the developing sporozoites.

A second question still unsettled is "What happens to the parasite in the first 4 days of the infection"? It appears evident that from the 5th day onwards the history of the schizont is a progressive development in size and number of particles of chromatin and that there is no release of merozoites until the full development is reached about the 8th to 10th days. In other words, the large fully mature schizont of the 9th day is the same individual seen as a small schizont on the 5th day and there is no increase in the number of forms between the commencement of schizogony and the release of merozoites. While more than one generation between sporozoite and mature schizont cannot be ruled out, the size of the schizont on the 5th day and its later history would suggest that each mature schizont is the end result of a single sporozoite which has entered a liver cell. Only visual observation of the earlier stages will settle this point and the quest should be interesting and not beyond solution.

An obvious question arises from a consideration of the sequence of events described in the development and maturation of the schizonts. Does the establishment of the blood infection terminate the exoerythrocytic phase, or does this persist, possibly as a low grade infection of the liver, to maintain the existence of the parasite in its host? This is a question already posed in the case of bird malaria when the pre-erythrocytic stage had been discovered (SHORTT, MENON and IYER, 1940) and now arising in mammalian malaria. It is a problem, the solution of which should be possible in the case of simian malaria with the knowledge now gained, and the importance of reaching a solution is evident on account of its possible bearing on the question of relapses. If certain of the merozoites resulting from exoerythrocytic schizogony enter fresh liver cells to maintain the local liver cycle, the destruction of the blood infection, either by specific immune response or by chemotherapeutic agents, would possibly leave intact the exoerythrocytic cycle which, under a suitable stimulus, could renew the blood infection.

A further point arises. In the case of avian malaria, two types of schizogony have been differentiated, one producing macromerozoites and one producing micromerozoites. The progeny of the latter are supposed to enter erythrocytes to produce the blood infection while those of the former are supposed to enter fresh cells of the reticulo-endothelial system to maintain the exoerythrocytic

cycle. Whether these observations are true or otherwise in avian malaria, our investigation, to date, of the pre-erythrocytic cycles of *P. cynomolgi* and *P. vivax* has given no indication of any such differentiation in the schizonts, and only further study will elucidate the point.

A feature of these liver infections, which is of considerable interest and which was probably one of the reasons why this cycle remained undiscovered for so long is their lack of infectiveness when transferred to clean animals. It will be recalled that all workers (HUFF and COULESTON 1947 MAMMALIAN MALARIA ENQUIRY 1948 SHORTT GARNHAM and MALANOS, 1948) failed to reproduce an infection of *P. cynomolgi* following the inoculation of suspensions of liver taken from animals in the incubation period of the disease. In this respect, *P. cynomolgi* behaves like *H. kochi* (GARNHAM 1948) and the genus *Haemoproteus* and unlike the avian exoerythrocytic parasites, which are always infective on subinoculation.

In conclusion we feel sure that the recent advances in our knowledge of avian and mammalian malaria, using the term malaria in a wide sense to include the pigmented blood parasites of birds, bats and monkeys, will raise questions of the taxonomic relationships of these various forms as well as of those found in cold blooded vertebrates. Some of the implications were briefly discussed by us recently (SHORTT and GARNHAM, 1948b), but the subject is beyond the scope of the present paper.

### SUMMARY

1. A description is given of experiments resulting in the discovery of the pre-erythrocytic stages in the life cycles of *P. cynomolgi* and *P. vivax*.

2. In the case of *P. cynomolgi* daily development of the forms commencing from the 5th day of the incubation period to the establishment of the blood infection on the 9th and 10th days is described.

3. In the case of *P. vivax* the forms described are those present on the 6th and 7th days of the incubation period.

4. Certain questions connected with immunity the mechanism of relapses the earliest forms of the pre-erythrocytic cycle, the non-infectivity of the pre-erythrocytic forms on sub-inoculation and the possible modifications in the taxonomic status of the plasmodia are discussed.

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# TRANSMISSION OF KALA-AZAR TO THE AUSTRALIAN MARSUPIALS *TRICHOSURUS VULPECULA* AND *PSEUDOCHEIRUS LANIGINOSUS*

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Visceral leishmaniasis or kala-azar is a disease chiefly confined to Eastern India, North China, the Mediterranean littoral, the Sudan and parts of tropical Africa and some regions in South America. In its distribution it closely follows that of the vectors, certain species of sandfly belonging to the genus *Phlebotomus* that habitually bite man. The disease is absent from Oceania and Australia, a fact which parallels the absence in these regions of the appropriate insect vectors. Nevertheless, it occasionally happens that persons suffering from kala-azar are landed in Australian ports, a happening that became much more frequent during the 1939-45 war when cases of this exotic disease, hitherto rarely seen in Sydney, began to make their appearance in metropolitan hospitals as the result of the greater number of Indian seamen.

Our thanks are due to Dr G A M HEYDON and Mr W K WHITTEN for their interest in the work, and to Professor E FORD for helpful advice and criticism in preparing the paper.

Cultures of leishmania used during the investigation were examined and maintained under the supervision of Mr A J BEARUP. Those obtained from India were sent by the courtesy of the Public Health Commissioner of the Government of India and of the Department of Pathology and Bacteriology, University, Calcutta.

The histological preparations were executed by G HAYDEN, and the photography was done by Mr S WOODWARD SMITH (Figs 1 to 4) and Mr H V GOLDING (Figs 5 to 14).

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entering the port. The observations here recorded resulted from this circumstance and the investigation was stimulated by the thought that the disease might well have become a diagnostic and even an epidemiological problem affecting allied personnel in some theatres of war even outside its normal range of endemicity. Therefore, it seemed desirable to establish the infection in experimental animals as a basis for further studies. In the absence of the hamster it was decided to test the susceptibility of the common phalanger or possum *Trichosurus vulpecula*, and it was shown that a pouch young of this marsupial became infected and developed typical lesions of leishmaniasis after inoculation with the flagellate form of *Leishmania donovani* from a culture derived from the blood of an Indian seaman affected with this disease (ARMY TAGE and BOLLIGER, 1945).

Following on this initial success, the course of the disease and the associated pathological changes were studied in a series of twenty five possums, including one ring-tailed possum (*Pseudocheirus longirostris*). Animals of varying ages, pouch young, adolescent, and fully grown, including some which had had their spleen removed, were used, and the inoculated material consisted of either the flagellate form of the organism in cultures of various ages or the non-flagellate form of leishmanias occurring in the blood or tissues of other infected animals or of a human.

#### TECHNIQUE

The possums used in this investigation were captured in gardens and parks of the suburbs of Sydney and in the neighbourhood of Moss Vale New South Wales. Some were bred in the laboratory. They were usually kept in individual cages about 3 by 3 feet in size. Their diet consisted of bread, lucerne, fresh fruit and vegetables, supplemented by leaves and twigs from eucalyptus, Moreton Bay fig and other native trees. This diet was apparently satisfactory as other possums lived on it for several years without showing any signs of malnutrition.

A saline suspension from cultures of kala-azar, fresh whole blood from infected animals, or material obtained by sternal puncture from a patient was injected intracardially or intraperitoneally. Kala-azar material was injected into each animal on one occasion only. In some animals, however, both the intracardiac and intraperitoneal routes were employed on this occasion. For intracardiac and occasionally for intraperitoneal injections the animal was anaesthetized with ether. In the case of pouch young, the mother was anaesthetized.

Blood for culture purposes was obtained by cardiac puncture or by means of a small cut in the external ear. A few drops of blood obtained under sterile conditions were transferred to a slope of N.N.N. medium. This culture was examined at approximately weekly intervals for the presence of flagellate organisms. A number of animals were killed after varying intervals following infection, while others were left to die from the disease or its complications.

Smears from fresh unfixed pieces of spleen and liver were prepared as soon as possible after death. These were stained with Leishman stain. Tissues for section were fixed in both formalin and Zenker solution and sections of a variety of organs were stained with haematoxylin and eosin, or Gierson and Giemsa stains. The great majority of sections, however, have been stained with haematoxylin-eosin after formal fixation. When deeply stained with haematoxylin the parasites can readily be demonstrated, often with good morphological detail, so that the more troublesome and less permanent Giemsa preparations are unnecessary particularly when the presence of infection has already been established by Leishman-stained smears and the identity of the bodies seen in the sections

was not in doubt. In any event, careful search of well stained haematoxylin sections will often reveal some parasites that are so favourably placed as to show both the nucleus and the rod-like kinetoplast.

## I NATURE AND COURSE OF THE DISEASE

### (a) GENERAL

After one single administration of *Leishmania donovani*, in the flagellate or non-flagellate form, the disease was definitely transmitted to well over 90 per cent of the inoculated animals (Table I). The course of the experimental infection was of a chronic progressive nature which, however, varied considerably as shown by survival figures of animals left to die from the disease itself. They varied from 81 days to 704 days (Table I). Several pouch young died from undetermined causes during the first month after inoculation. These non-specific deaths have not been included in our series.

Generally speaking, pouch young surviving the first months after injection showed retardation of development which was evidenced by a longer stay in the pouch up to 6 months instead of the usual 3 to 4 months, stunted growth and fine gray woolly fur.

On the other hand, some of the larger and fully grown animals which survived infection for more than a year showed no immediate ill effects from the inoculation for several months, apart from a transient conjunctivitis and other eye changes which will be discussed later. Loss of weight or unsatisfactory increase in body weight of the growing animal was one of the earlier signs of the presence of the kala-azar infection. The fur also showed changes inasmuch as it frequently developed a fine woolly structure. In a few instances necrotic skin lesions appeared temporarily on or near the root of the tail.

In the latter stages the testicles of the males and the pouches of the females atrophied markedly. This and spermatogenesis will be discussed separately.

In the earlier stages, moderate and transient inco-ordination of gait and nervous hyper-irritability may also be noted. Later on, tetanic spasms or clonic convulsions or athetoid movements may also occur. Polyphagia is now also a marked feature, but in spite of it, loss of weight is very marked and may or may not be accompanied by eye lesions or neurological symptoms.

A marked anaemia becomes apparent in the later stages and is severe in the terminal phases. Haemoglobin values as low as 2.8 grammes per cent were found in Possum 349 (normal values 16-17 grammes per cent).

### (b) EYE CHANGES

Frequently, the first signs of the infection were found in the eye. Unfortunately, however, in the earlier part of this work, the eyes were not examined, but in the last twelve infected specimens it became evident that definite and usually serious eye changes occur in the majority of possums infected with kala-azar.

TABLE I.

Number	Sex.	Body weight on infection. Kg	Body weight at death. Kg	Maximum body weight recorded. Kg	Survival period. Days.	Type of material injected.	Mode of infection
309	F	48	1.29	45	134	Blood from 81	p
333	F	2.32	1.77	2.45	257 h.	Culture—T rat perle	i.c.
341	F	2.1	1.8	2.1	244	*Culture—Human	ap
348	F	1.7	1.1	1.1	81	Blood from 418	p
408	ML	2.2	1.7	2	94	Blood from 61	c, ap.
409	ML	1.7	1.8	2.1	309 h.	Culture—Human	c, ap.
410	ML	2.0			104 h.	Blood from 409	i.c. p.
411	ML	1.4	1.2	1.4	104 h.	Blood from 409	ip.
415	ML	1.8	1.3		140 h.	Culture—T rat perle	ap
421	F	0.42	0.7		40 h.	Culture—Human	p
53	F	0.06	1.4		15 h.	Blood from 61	l.c., p
423	F	1.9	1.78	2.0	181 h.	Culture—Human	p
536	F	0.04	0.036		60	Culture—T rat perle	p
443	F	0.85	1.23	1.8	318	Blood from 430	p
605	ML	2.3	2.3		31 h.	Culture—T rat perle	l.c.
606	ML	2	1.9	2.5	326 h.	Culture—Human	p
611	ML	0.09	1.6	2.0	106	Culture—Human	p
612	ML	1.76	1.5	1.74	23 h.	Culture—Human	ap
613	ML	0.05	0.39	0.39	70 h.	Culture—Human	ap
615	ML	0.8	1.37	3	267 h.	Marrow—Human	ap
617	ML	0.06	1.7		271 h.	Culture—Human	ap
628	ML	0.03	1.3	1.6	479	Culture—Human	p
630	ML	0.1	1.8		0"	Culture—Human	ap
641	ML	0.07	0.73	0.8	21	Culture—Human	p
1003 R	F	0.6	0.43		4	Culture—T rat perle	p

1003 R = Ringtailed possum *Pseudocheirus peregrinus*. i.c. = Intracardiac

h. = Killed.

— Culture 10 months old

ip = Intraperitoneal.

The signs of these in the living animals were as follows as early as 1 to 6 days after the experimental infection, varying amounts of lachrymation, photophobia, hyperaemia and secretion of pus were noticed in one or both eyes. In some instances the exudate was so copious as to justify a diagnosis of purulent conjunctivitis. The exudate, however, has not yet been examined bacteriologically. This condition cleared up usually within a few weeks or months, but was then followed in some cases by a slight but distinct haze over an inconstant portion of the cornea, which became more pronounced as time went by. At this latter stage an iritis also developed as indicated by the irregular margin of the iris.

On examining such eyes with a magnifying glass under oblique focal illumination, small whitish dots could be seen adhering to the posterior aspect of the cornea. These "keratic precipitates," though very small and comparatively few at first, increased in number as the disease progressed, and in advanced cases they reached pinhead size. Increasing keratitis, giving the cornea ultimately a bluish white opaque appearance, soon made it almost impossible to see the pupil, and severely or completely impaired the sight of the animal. In other instances, iritis of varying degree in one or both eyes was the primary lesion developing after a conjunctivitis. Following the instillation of a mydriatic into the eyes, it became obvious that adhesions of the iris to the anterior lens surface were present and some of these adhesions were seen to break down when the pupil was dilated. Subsequently, keratitis was also seen to develop in these eyes. In other instances, corneal abrasions, scars and haemorrhages into the iris were noted before or simultaneously with the development of the keratitis or iritis or both.

So far seven animals have been observed to become blind. Some of the others in our present series may have suffered the same fate, had they been permitted to live sufficiently long. Following obvious impairment of sight, the eyeballs shrank considerably, even in the absence of marked keratitis.

#### (c) EFFECTS ON SPERMATOGENESIS, TESTICLES AND POUCH

Advanced kala-azar produces marked degenerative changes in the testicles. This manifests itself in the living animal by a diminution in the size of the testicles and by alterations in spermatogenesis. The latter becomes evident on examining the spermatozoa in a drop of urine (BOLLIGER, 1942). Pathological forms may be seen before any noticeable alterations in the size of the testicles occur. These abnormal spermatozoa may have lost their head or may possess two heads or an abnormally shaped head or vacuoles in the neck, body or tail.

In the advanced stages, spermatogenesis ceases completely and the testes are only half or a third of the normal size. Some males infected at a sexually immature stage never produced spermatozoa. The testes remain undersized and may be less than one-fourth normal size. On palpation the normally large carrot-shaped prostate has practically disappeared.

In the majority of females examined, the pouch and the mammary glands and nipples contained in it were found to atrophy as soon as the disease was well established. In advanced disease the pouch was only about a third as deep and wide as that seen in uninfected controls of similar age. Ultimately the pouch presented itself as a structure of about one-third the usual length, with the lips separated and with grossly atrophied mammary glands and nipples.

#### (d) BLOOD CULTURES.

The principal diagnostic method employed was the blood culture. A few drops of blood were transferred on to N.N.N. medium. The flagellate form of leishmania always developed when the blood specimen was obtained from an animal with advanced kala-azar. However in the present investigation, no attempt was made to ascertain just how soon after inoculation a positive culture could be obtained. A positive culture was obtained 45 days after inoculating Possum 349. On the other hand, repeatedly negative cultures were obtained from animals which were not markedly ill and which, on histological examination, proved to be infected with the disease. In other instances, a negative culture became positive after a few months. This was the case in Possum 409 though only very few parasites were found to be present in spleen and liver. Probably fewer negative cultures would have been obtained had the blood been concentrated prior to transfer to N.N.N. medium, as recommended by NAPIER (1946) and others.

### II AUTOPSY FINDINGS.

The weights of the organs removed at autopsy are summarized in Table II. In addition, the following points will be elaborated:

- (a) General macroscopic and microscopic appearance of spleen, liver, kidney, central nervous system and eye.
- (b) The histology of all the organs examined of three illustrative cases together with a short history of the experimental illness.
- (c) The macroscopic pathology of the eye and central nervous system in further individual cases.

#### ( ) GENERAL MACROSCOPIC AND MICROSCOPIC APPEARANCE.

##### SPLEEN

**Macroscopic.**—In the fully grown normal *Trichurus vulpecula* the spleen is a tri-lobed structure weighing from 3 to 5 grammes. In the infected possums the weight of the spleen varied from a fraction of the above maximum to nearly six times the maximum.

Excluding one from a very immature animal, three spleens were grossly atrophic, being represented by a thin translucent pink fibrous band. Of the enlarged spleens more than half were grossly enlarged and generally presented a smooth dark fleshy appearance. Some showed varying degrees of nodularity, having irregular translucent areas, pale spots or irregular patches visible through the capsule or showing as discrete pale nodes on cross section. (Figs. 1 and 2.)

**Microscopic.**—The atrophic spleens showed markedly thickened capsules, dense fibrous trabeculae and a cellular picture that differed according to the type of the disease.

TABLE II

Number	Body weight at death Kg	Spleen Grammes	Liver Grammes	Average weight of kidney Grammes	Average weight of adrenal Grammes	Prostate Grammes	Average weight of testis Grammes	Average weight of ovary Grammes
309	1.39	Sp	60	7.5	0.24			0.04
333	1.77	7.0	56	10.0	0.17			0.18
341	1.5	16.3	76	6.9	0.42			0.042
349	1.1	1.85	22.5	5.3	0.11			0.045
408	1.7	19.0				12.0		
409	1.9	29.0	85	7.1		1.0		
410	1.8	25.0	73	10.0				
411	1.2	5.6	60	11.8		0.3	0.3	
415	1.55	Sp	51	8.2	0.12	3.0	2.1	
531	0.7	4.0		3.8				
532	1.4	8.3	56	6.0				0.07
533	1.7	11.5	64	7.0				0.07
536	0.056	0.3	1.73	0.3				
543	1.23	4.8	29.2	6.4	0.25			0.062
605	2.3	7.5	67					
606	1.0	12.0				0.0		
611	1.57	17.1	93	7.7		3.5	3.4	
612	1.25	8.0	45	6.2	0.09	0.25	0.7	
613	0.39	9.0	26	3.3		0.15	0.14	
625	1.37	2.0	42	7.0		1.2		
627	1.7	9.1	66	14.5		0.17	0.8	
628	1.3	1.25	37	6.8		1.6	0.8	
630	1.8	14.5	72	7.8	0.13	2.2	1.1	
641	0.73	11.0	53	7.0	0.11	0.25	0.14	
1003 R	0.43	3.47	36	3.0	0.05			

Sp = Splenectomy prior to inoculation

In Animal 349, which survived 81 days, there was very pronounced endothelial hyperplasia with large swollen parasitized cells dominating the field (Fig 3). In the other two atrophic spleens (470 and 657 days) proliferated endothelial cells were largely replaced by plasma and lymphoid cells, the parasites having disappeared from one case and become very scanty in the other. The enlarged spleens also present a varied picture according to the stage of the disease. There are often large areas of swollen, heavily parasitized, endothelial cells (the "clasmatocyte tissue" of MELENEY, 1925), while in other parts of the section or in other spleens at a later stage these are interspersed with plasma cells, or later again with large lymphocytes and fibroblasts, the parasites becoming progressively more scanty. This is well illustrated in Animal 409.

## LIVER

*Macroscopic*—In the infected animals the size of the liver varied from pathologically small specimens to excessively large ones and, generally speaking, those individuals with a small spleen had also a small liver. In the majority of experiments, however, the size



of the liver fell within normal upper limits. As shown in Table I, the weight of this organ in infected phalangers of more than 1 year of age varied from 22 to 83 grammes but only in two instances did the liver exceed 80 grammes weight definitely above normal.

In appearance the livers were of pale red or yellow red colour. Some presented an almost even surface while others were mottled. Again, others contained numerous small diffuse haemorrhagic areas. In the case of the two splenectomized animals, the liver was characterized by smooth surface and weight within normal limits.

*Microscopic*.—Some livers presented fairly advanced fatty or granular degeneration of the parenchyma cells. In general, the striking change was the classical proliferation of the Kupffer cells of the sinusoids which were as a rule heavily paritized and accompanied by varying degrees of plasma cell and lymphocytic infiltration (Fig. 4). This process, as in the spleen, increases as the disease progresses and tends to obscure or replace the hyperplastic endothelium, in which also the parasites become progressively fewer. Paritized parenchyma cells were not seen.

#### KIDNEY

*Macroscopic*.—The kidneys were pale in colour but otherwise the majority presented fairly normal appearance. The kidneys of Possum 411 and 627 (Table II) were strikingly enlarged. There were small haemorrhages on the surface of the kidneys of Possums 411 and 841.

*Microscopic*.—As indicated in Table III, more than half of the possums showed parasitic invasion of the kidney. The lesions consisted of focal proliferation of interstitial connective tissue cells and round-celled infiltration in or around these areas, either intertubular or subcapsular in situation. The vascular endothelium of the glomeruli frequently showed paritized cells. In some instances there were well marked degenerative changes in the renal epithelium and areas of capillary haemorrhage were not infrequent. In general the parasites were confined to vascular or connective tissue elements, but in at least one instance they were clearly within the cells of the renal tubules (Possum 637 Fig. 5).

#### CENTRAL NERVOUS SYSTEM

*Macroscopic*.—In the majority of experiments no obvious changes had occurred. In one instance however (Possum 543), an extensive subdural haemorrhage was noted. In another animal (Possum 628) there was thickening of the dura mater.

*Microscopic*.—In the brain and spinal cord the lesions take the form of more or less dense invasion of the pia-arachnoid, with lymphoid cells and perivascular infiltration extending into the cerebral cortex or the grey matter of the cord. There is marked proliferation of adventitial cells around the cortical capillaries, and many of these are paritized (Figs. 6 to 18).

#### ETES.

*Macroscopic*.—The gross changes in the eye have already been described in the section dealing with the course of the disease.

*Microscopic*.—In the infected eyes, histological changes may be found in the cornea, which may be thickened and oedematous and vascularized by small adventitious capillaries around which are proliferating endothelial cells, many containing leishmanias (Figs. 11 and 12). Adhering to the posterior surface are often agglomerations of lymphocytes and other mononuclear cells, representing the keratic precipitates to be observed during life. The ciliary body is consistently infiltrated with lymphoid and plasma cells, and occasional paritized macrophage cells are to be seen (Fig. 13). In some instances the whole uveal tract is attacked, showing vascular engorgement, round-celled infiltration, and many phagocytic cells containing parasites or choroidal pigment or both. In one case the ganglion cell layer was invaded by pigmented macrophages, some of which contained leishmanias (Fig. 14). Where the optic nerve has been included in the section it has in two cases been found to show proliferation of perivascular cells in the connective tissue sheath, accompanied by lymphocytic and plasma-celled infiltrations and varying number of paritized cells.

TABLE III

Possum number	Spleen	Liver	Kidney	Brain	Eye	Pituitary	Adrenal	Ovary	Fetus	Prostate gland	Heart	Lung	Alimentary canal	Paracloacal glands	Pancreas	Typical histology
301	Sp	+	+	—	—	—	+	+						+	+	Yes
333	+	+	+	+	+	—	—	—				—			+	Yes
341	+	+	+	+	—	+	+	—			—			—		Yes
349	+	+	+	—	—	—	+	+				+	+	—		Yes
408	+	+	—	—	—	—	—	—	—	—						Yes
409	+	+	+	—	+	—	—	—								No*
410	—	—	—	—	—	—	—	—								No***
411	+	+	+	—	—	—	—	—	—	—						Yes***
415	Sp	—	—	—	—	—	—	—	—	—		—	—			No**
531	+	+	—	—	—	—	—	—								Yes
532	+	+	+	—	—	—	—	+								Yes
533	—	—	—	—	—	—	—	—								Not
536	+	+	—	—	—	—	—	—								Yes
543	—	+	+	+	+	+	+	—								Not†
605	—	—	+	—	—	—	—	—								Yes
606	+	—	—	—	—	—	—	—		—						Yes
611	+	+	+	—	—	—	—	—								Yes
612	+	+	+	—	—	—	—	—								Yes
613	+	+	—	—	—	—	—	—					—			Yes
625	—	+	+	+	+	—	—	—								Yes
627	+	+	+	—	—	—	—	—	—	—						Yes
628	+	+	—	+	+	—	—	—	—	+						Yes
630	+	+	+	+	+	+	+	—	+					+		Yes
641	+	+	+	+	+	—	+	—	—				—			Yes
1003R	+	+	+	+	+	+	—	—						+		Yes

+ Leishmania seen in section

— No leishmania seen

Blank No section examined

Sp Splenectomy prior to inoculation

\* Histology atypical owing to marked plasma cell reaction

\*\* Marked plasma cell reaction in liver, some endothelial hyperplasia Well marked eye lesion regarded as leishmanial

\*\*\* Both animals inoculated with blood from 400 Possum 410 killed after 108 days No conclusive evidence of infection

† Suggestive lesions in liver and kidney

†† Lived 31 days only, but showed round-celled infiltration in kidney and some leishmania in interstitial tissue



## (b) THE HISTOLOGY OF THREE ILLUSTRATIVE CASES, TOGETHER WITH A SHORT HISTORY OF THE EXPERIMENTAL DISEASE

## POSSUM 349

## HISTORY

- 28.2.47 Fifteen ml of blood obtained from Possum 415 (inoculated with kala-azar 180 days previously) injected into almost fully grown female 349. Body weight, 1.7 kg.
- 1.3.47 Eyes covered with purulent exudate. Definite keratic spot on the lower margin of the left pupil.
- 8.3.47 Spot on left eye still visible.
- 14.4.47 Blood culture positive for leishmania.
- 18.4.47 Purulent conjunctivitis in both eyes. Pupils large and irregular. Spot on left eye still visible. Animal losing weight rapidly.
- 19.5.47 Body weight, 1.1 kg. Comatose. Died a few hours later. Haemoglobin content of blood, 2.8 grammes per cent.

## POSTMORTEM EXAMINATION

All the organs were pale in appearance. The spleen, weighing 1.85 grammes, consists of a thin pale pink translucent band. The liver weighs only 22.5 grammes. A yellow gelatinous deposit, consisting mainly of fibrin and leucocytes, was found principally in the following three sites, *viz.*, between the posterior aspect of the eyeball and the bony walls of the orbit, on the floor of the anterior cranial fossa extra-durally, and on the fascial condensations surrounding the female urethra. At the superior end of the fascial condensations about the urethra a small tumour weighing 0.3 gramme was noted.

## Histology

**Spleen**—The capsule is markedly thickened and the pulp is intersected with numerous very thick fibrous trabeculae. The lymphoid follicles are few and atrophic and the pulp is composed almost entirely of large endothelial cells most of which are heavily parasitized by leishmania (Fig. 3).

**Liver**—There is gross hyperplasia of the Kupffer cells, practically all of which are heavily parasitized and form conspicuous islets among the liver cells. There is little or no lymphocytic or plasma cell infiltration in this section. The picture is one of almost pure endothelial hyperplasia (Fig. 4).

**Kidneys**—Both show considerable degeneration of tubular epithelium, particularly in the subcapsular region and more markedly in the left kidney. There is no appreciable round-celled infiltration and very little increase in the interstitial connective tissue, but here and there are occasional swollen parasitized cells between the tubules and in a few of the glomeruli.

**Suprarenal**—No gross histological change is noted but groups of parasitized cells are to be seen in the outer zone of the cortex.

**Ovary**—In the germinal layer among the primary follicles are occasional parasitized interstitial cells.

**Pituitary**—Only a small fragment of gland tissue present in section. No leishmania seen.

**Lung**—Some endothelial hyperplasia with resulting thickening of alveolar walls which show fairly numerous parasitized cells.

**Heart**—Muscle is relatively normal. No increase of cells in connective tissue septa. No leishmania seen.

**Caecum**—Some desquamation of the mucosa is present. There is also some endothelial proliferation and round-celled infiltration of the submucous tissues and occasional parasitized cells are seen.

**Intestine**—Occasional parasitized cells are present in the submucous connective tissue.

**Brain**—Portions of olfactory lobe and fore brain, cerebellum and medulla oblongata show no significant changes. No leishmania seen.

**Eye**—Shows relatively normal histology. No leishmania seen.

## POSSUM 409

## HISTORY

- 15.5.45 Culture prepared from blood of Indian seaman suffering from kala-azar was injected into the peritoneal and cardiac cavity of nearly fully grown male body weight, 1.8 kg
- 6.6.45 Blood culture negative for leishmania and formal gel test negative.
- 18.12.45 Body weight, 2.1 kg. Blood culture positive, formal gel test positive. Shortly afterwards it was noticed that the animal began to lose its sight, the cornea showing a bluish white opacity
- 19.3.46 Body weight, 1.94 kg. General health, apart from loss of eyesight, was fairly good and the animal had ravenous appetite. Animal killed.

## POSTMORTEM EXAMINATION

The spleen, dark red fleshy organ with smooth surface, weighed 29 grammes and was about seven times its normal size. The liver was also large weighing 85.5 grammes. The prostate was only about fifth of its maximum normal size weighing 1.0 grammes.

## Histology

*Spleen*.—The normal architecture is lost. Although only occasional trophic lymphoid follicles can be seen the bulk of the cellular part of the organ seems to be composed of lymphoid and plasma cells, obscuring background of endothelial cells in which very occasional bodies resembling poorly stained leishmania can be made out. The capsule is thickened and the fibrous trabeculae throughout the organ are somewhat increased.

*Liver*.—Presents striking picture of sharply defined foci of round-celled infiltration either periportal or intralobular. The cells of these foci appear to be lymphocytes and plasma cells, and in few situations where they are less densely crowded some groups of swollen Kupffer cells can be seen. After long search several such cells containing few leishmania were found. The liver cells are granular and the cytoplasm stains rather poorly.

*Kidney*.—Shows numerous round-celled foci similar to those seen in the liver and occasional interstitial cells with poorly staining leishmania.

*Eye*.—The cornea is thickened and vascularized and shows marked proliferation of endothelium of adventitious capillaries and some lymphocytic infiltration. The ciliary body is densely infiltrated with lymphocytes and plasma cells, as is also most of the choroid coat. Occasional parasitized cells were found after prolonged search.

*Note*.—Apart from its blindness this animal was in relatively good condition when killed. This, together with the appearance of the cellular reaction in the liver and spleen and the paucity of parasites, suggests an attempt at spontaneous cure.

## POSSUM 630.

## HISTORY

- 11.6.45. Male, pouch young about 3 inches long and weighing about 0.1 kg., was injected with the water of condensation of culture prepared from the blood of an Indian seaman suffering from kala-azar (culture was prepared 23.5.45). Subsequently animal seemed to develop quite normally and reached maturity within 1 year.
- 22.7.46. Numerous sperms, many of which are pathological in appearance, were seen in the urine.
- 21.10.46. Body weight, 2.16 kg
- 4.11.46. Body weight, 2.05 kg
- 19.11.46. Blood culture positive for leishmania.
- 3.12.46. Body weight 2.05 kg. No eye change, health.
- 17.12.46. Urine contains motile but mostly dead to lose weight.

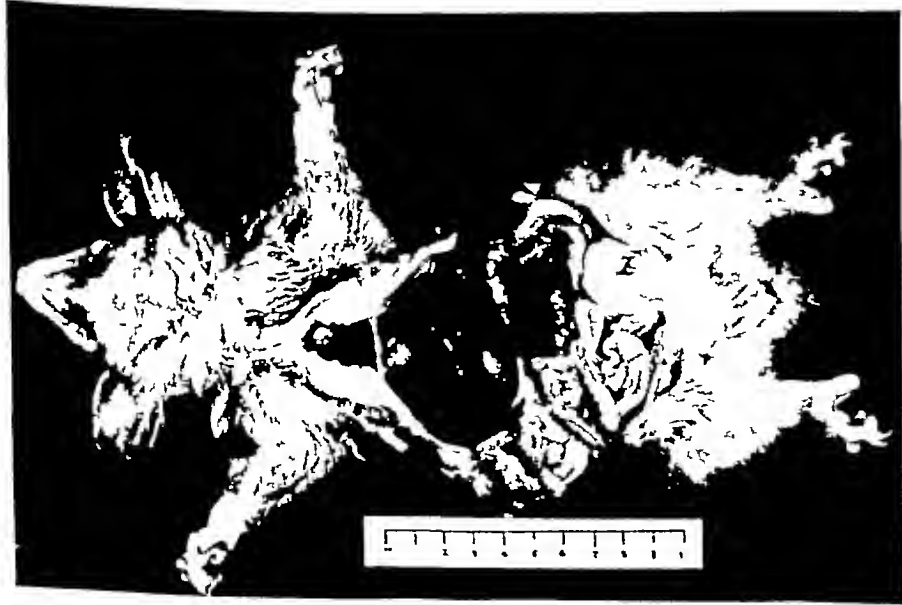


FIG 1. Possum 613. Adolescent. Killed 70 days after inoculation with a culture of *Leishmania*. Note the large mottled spleen and liver.

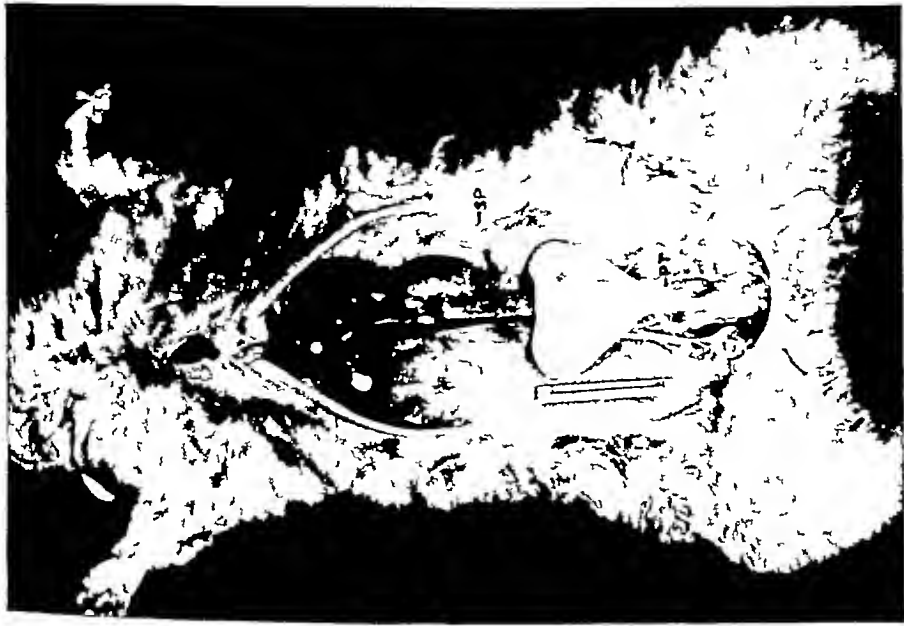


FIG 2. Possum 628. Died 470 days after inoculation with *Leishmania*. Note small thin translucent trilobed spleen placed near kidney, small liver, small testes and small rudimentary prostate, being about one tenth of its normal size. SP—Spleen. PT—Prostate.



FIG. 3. Parasite 349. Spleen showing heavily parasitized endothelial cells of pulp.  $\times 600$ .

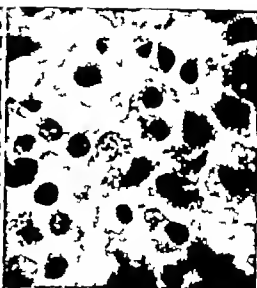


FIG. 4. Parasite 349. Liver heavily parasitized cells of sinusoid.  $\times 600$ .



Parasite 627. Shows *Leishmania* in cells of renal tubules.  $\times 400$ .



FIG. 6. Parasite 543. Brain. Infiltration of parasitophorous cells. Peri-capillary infiltrations in cortex.  $\times 400$ .



FIG 7 Possum 543 Brain Cellular hyperplasia and infiltration amongst nerve cell layer in mid brain  $\times 150$

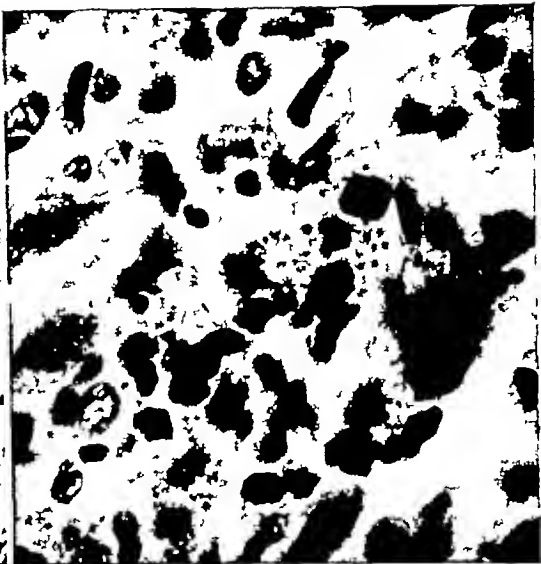


FIG 8 Possum 543 Portion of Fig 6 enlarged to show parasitized cells  $\times 800$

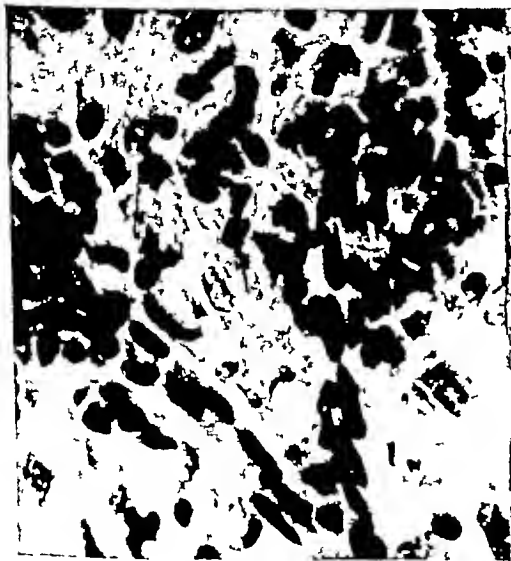


FIG 9 Possum 625 Brain Shows perivascular infiltration of cortical capillaries *Leishmania* can be seen in some of the cells  $\times 800$

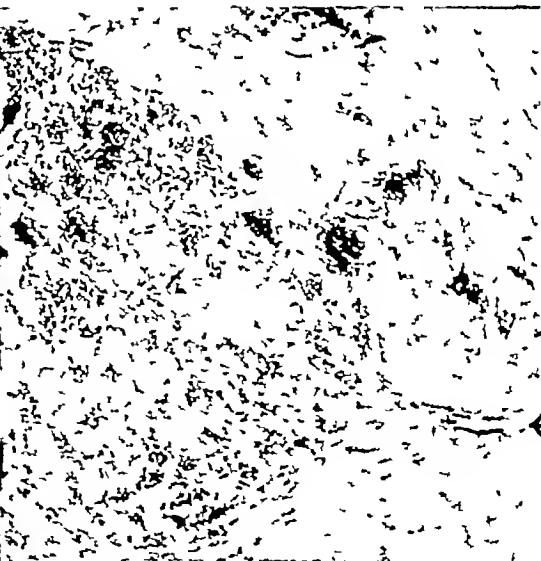


FIG 10 Possum 335 Section of spinal cord in cervical region showing granulomatous areas marked in grey matter. The arrow shows the position of the central canal  $\times 50$





FIG. 3. Possum 349. Spleen shows g. has the parasitized endothelial cells of pulp. 600.

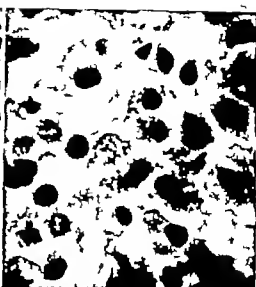


FIG. 4. Possum 349. Liver heavily parasitized cells of sinusoids. x 600.



FIG. 5. Possum 627. Shows *Leishmaniae* in cells of renal tubules. 940.



FIG. 6. Possum 543. Brain. Infiltration of parasitoid. Peri-capillary infiltrations in cortex. 56 x.

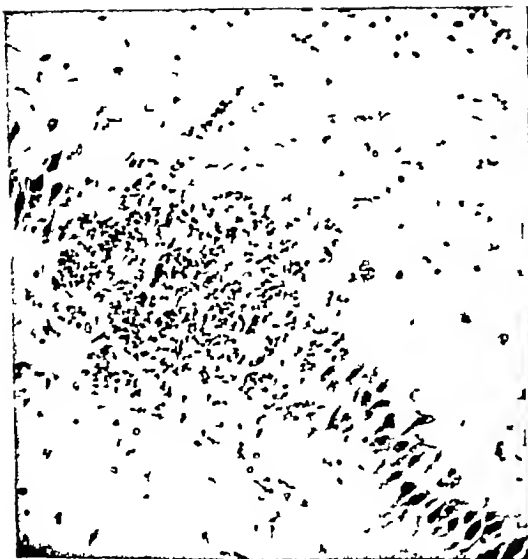


FIG 7 Possum 543 Brain Cellular hyperplasia and infiltration amongst nerve cell layer in mid brain  $\times 150$



FIG 8 Possum 543 Portion of Fig 6 enlarged to show parasitized cells  $\times 800$

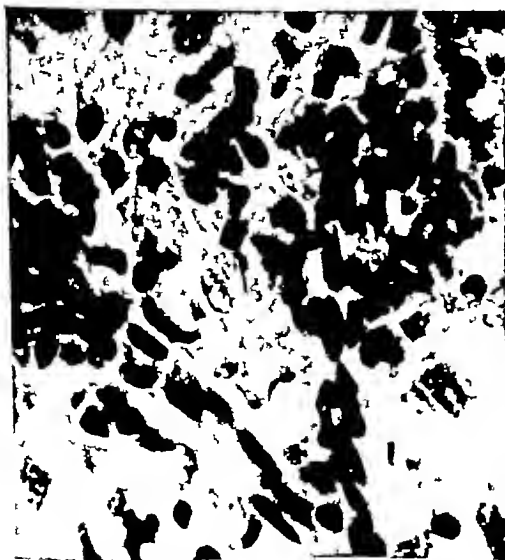


FIG 9 Possum 625 Brain Shows perivascular infiltration of cortical capillaries *Leishmania* can be seen in some of the cells  $\times 800$

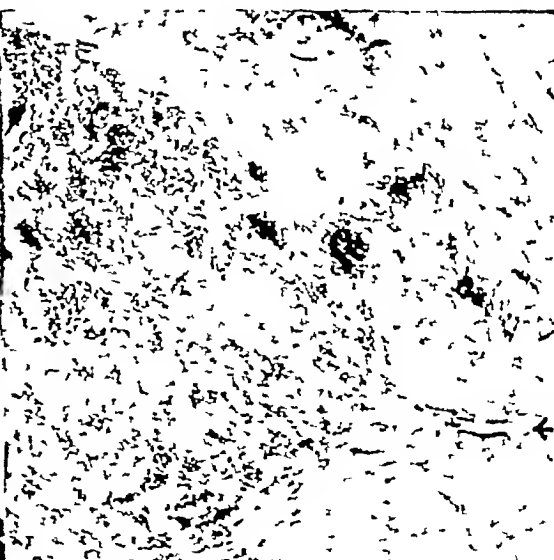


FIG 10 Possum 333 Section of spinal cord in cervical region showing granulomatous areas most marked in grey matter The arrow shows the position of the central canal  $\times 50$

*Right eye.*—The cornea appears thinned and the conjunctival epithelium only a few cells thick. There is some slight vascularization at the margins.

The ciliary bodies are densely infiltrated with lymphoid and plasma cells. No parasitized cells are seen. Some cells are adhering to Descemet's membrane. The optic nerve shows numerous areas of cell proliferation about the capillary vessels. These appear to be chiefly cells of the endothelial type or fibroblasts forming granulomatous areas about the capillary vessels. Very occasional faintly stained parasites seen.

*Left eye.*—There is dense lymphoid and plasma cell infiltration of the ciliary body, many aggregations of cells adhering to the Descemet's membrane, including lymphocytes, plasma cells, few polymorphonuclear leucocytes and fairly numerous mononuclear phagocytic cells containing choroidal pigment. No leishmanias seen. The conjunctiva and cornea appear thinned and atrophic, and slightly vascularized at the margins.

#### POREUM 413.

*Central nervous system.*—No notable change in brain cortex.

*Eye.*—Both eyes show marked vascularization of the cornea and round-celled infiltration about the adventitious vessels. The ciliary bodies are densely infiltrated with lymphoid and plasma cells. No leishmanias seen.

#### POREUM 423

*Central nervous system.*—The meninges are engorged and infiltrated with lymphocytes. Various portions of the brain cortex and cerebellum show gross cuffing of the capillaries which are surrounded by lymphoid and adventitial cells, many heavily parasitized by leishmanias (Fig. 9).

*Eye.*—There are gross lesions present consisting of aggregations of parasitized cells in the optic nerve between the nerve fibres. There are similar cell infiltrations in the adjacent sclera. The iris and ciliary body are densely infiltrated with lymphoid and plasma cells (Fig. 13) and the whole uveal tract is more or less infiltrated. The cornea shows marked vascularity and the capillaries are surrounded by cells containing numerous parasites.

#### POREUM 623

*Central nervous system.*—The dura mater is thickened and infiltrated, and shows many numerous leishmanias. The cerebral cortex shows marked perivascular infiltration and proliferation of adventitial cells, many of which are parasitized. The pial vessels of the medulla oblongata and the capillaries of the brain substance show remarkably broad perivascular infiltrations and proliferations, with many parasites.

*Eye.*—The cornea shows oedema and marked vascularization. Foci of cell infiltration and aggregations of endothelial cells and fibroblasts are situated about the adventitious capillaries. There are many parasitized cells in these (Figs. 11 & 12). The ciliary body and the uveal tract generally are infiltrated, the pigmented coat being largely disorganized and showing widely dispersed pigment-bearing macrophages. The vitreous layer with its cells and the ganglion cell and nerve fibre layer of the retina shows some infiltration by macrophage cells some of which contain leishmanias.

#### POREUM 641

*Central nervous system.*—The mid-brain shows no vascular changes or infiltrations. Over the cerebellum there is some round-celled infiltration and endothelial proliferation about the pial vessels and here also there are foci of parasitized cells. None are seen in the brain substance.

*Eye.*—The cornea is rather thickened but shows no vascularization or cell infiltration. The ciliary body is infiltrated with plasma cells and lymphocytes, with some large parasitized macrophage cells. There is some endothelial proliferation in the perocular tissues in the region of the optic nerve and some parasitized cells are present in the optic nerve itself.

POSSUM 1003 R (*Pseudocheirus lamginosus*)

*Central nervous system*—The fore-brain is unaffected. The mid-brain substance is relatively normal but the choroid plexus shows many parasitized endothelial cells. The cerebellum is unaffected. Also the medulla and spinal cord are not affected.

*Eye*—The cornea is relatively normal and no vascularization or infiltration is seen. The ciliary body is infiltrated with lymphoid and plasma cells, and here and elsewhere in the choroid coat are fairly numerous parasitized endothelial or macrophage cells.

## DISCUSSION

In twenty-two out of twenty-five inoculated Phalangeridae, leishmania were recovered in one or more internal organs (Table III). In Animal 415, where no parasites could be found, an advanced eye lesion was present and the blood of this animal inoculated into Phalanger 349, produced a typical leishmaniasis in the recipient. On these grounds, 415 must be considered an infected animal. Specimen 410 was inoculated with 3 c.c. of blood obtained from Possum 409 which, at the time, was considered to be undergoing an attempt at spontaneous cure because of the paucity of parasites, atypical cellular reactions in liver and spleen, and good general condition. Animal 410 was killed accidentally after living for 108 days after inoculation. The spleen was found to be enlarged, and a characteristic haze over the cornea suggesting an early keratitis was present. However, no parasites or typical histology were found in this animal. Possum 411, which was injected with a larger amount of blood (7 c.c.) from Possum 409, developed the disease in its typical form. Although no parasites could be found in Phalanger 533, suggestive pathology was present in spleen and liver.

Summing up, it is evident that the typically Australian marsupial *Trichosurus vulpecula* and, in all probability, also *Pseudocheirus lamginosus*, are highly susceptible to experimental kala-azar infection. In the absence of the hamster (*Cricetus griseus*), we have in Australia a highly suitable animal, the possum, for laboratory investigations of leishmaniasis, and the possibility should not be overlooked that, under appropriate conditions, *T. vulpecula*, or some other marsupial, might become the animal reservoir for leishmania.

In the present series of experiments no difference was observed between the infectivity of cultures, blood, or bone marrow. Also a culture injected into Possums 341 and 641, which had been subcultured eight times over a period of 10 months, was found to be still as virulent as a fresh culture.

The first noticeable sign of the experimental disease is a conjunctivitis observed as early as one or several days after the intraperitoneal inoculation with leishmania. This is noteworthy since the incubation period of kala-azar is assumed to be at least several weeks. At this stage, however, no explanation of the production of this lesion can be given. In general, the frequency of ocular, as well as of nervous manifestations noted, was unexpected in view of their absence in human leishmaniasis. This aspect, however, will be discussed in connection with the histology of these lesions.

Splenomegaly which characterizes the human disease, occurs in the majority of the infected *Phalangeridae* (Table II) and the surprising occurrence of some abnormally small spleens has been observed previously in infected dogs (ADLER and TIMODOR, 1934).

The severe atrophy of the genital organs, particularly in the male, may be due to the direct action of the parasite on the germinal epithelium. However in only one instance could parasites be found in the testicles. On the other hand, the pituitary gland has been found smaller than usual and frequently infected with leishmania (Table III). Thus interference with the secretion of gonadotrophins may well have happened. This, in turn, may have produced the genital atrophy.

In a similar manner the severely stunted growth of many of the experimental animals could be explained by a lack of growth hormone secretion from the pituitary.

Furthermore, the adrenals have also frequently been found to be infected and smaller than usual, and therefore adrenal cortical insufficiency may at least be a contributing factor to the atrophy of genital organs.

However leishmania may occur in almost any organ of an infected *T. ulpecula* or *P. lewingsi* (Table III) and the histological changes have been described in protocols of illustrative cases. In general, they were identical with those which have been communicated in the literature by CHRISTOPHERS (1904) SNOWY (1923), MELLOR (1925), HU (1933), and others, with the exception of those appearing in the central nervous system and the eyes. In twelve animals in which sections from various parts of the brain were studied, gross changes of a granulomatous nature and parasites were found in seven. In thirteen animals the eye was examined. Of these eight showed gross histological changes with the presence of parasites, while another one showed similar tissue changes but without demonstrable parasites.

Of eleven animals in which both brain and eye were examined, seven showed typical lesions in both situations. In the eighth case (Possum 415) of this series, typical tissue changes without demonstrable parasites were present in the eye, while the brain appeared normal. In the remaining three, in which the brain and eye were examined, no lesions or parasites were seen.

In human kala-azar the nervous system is rarely attacked. Thus, NAPIER (1946) writes "The nervous system seems to be peculiarly free from attack by the parasites or their toxins. The mental condition is always quite clear even in the final stages and delirium is less common during pyrexial attack in this disease than in any other."

Nevertheless, some writers refer to the occasional nervous manifestations. BRAHMACHARI (1928) says "Tetany epileptiform fits retinal and meningeal haemorrhages are unusual complications." This writer also mentions cerebral haemorrhage and spinal meningitis in association with infantile kala-azar and refers to CHRISTOPHERS (1904) finding of leishmania in petechiae of the arachnoid in Indian kala-azar.

Similarly, eye lesions in the human disease have received scant mention, although the literature contains occasional references to conjunctivitis and retinal haemorrhages DUKE-ELDER (1937), in referring to the "very rare leishmania keratitis," based his brief note on the paper of CHAMS (1929) which, however, has not been available to the writers of the present communication. ELLIOT (1920), in his treatise on tropical ophthalmology, makes no mention of leishmanial eye lesions. Therefore, it would seem that central nervous and eye manifestations in human kala-azar are rare complications. In the naturally occurring disease in dogs, on the other hand, keratitis is sufficiently common to have attracted the attention of various observers. LEMAIRE, SERGENT and L'HERITIER (1914) give a good description of the histological appearances in such lesions occurring in dogs in Algiers. ADLER and THEODOR (1934) describe eye changes in dogs experimentally infected with *Leishmania donovani*. These appear to have been confined to the cornea and corneo-sclerotic junction and consist of infiltrations with macrophages and plasma cells. The condition of the uveal tract is not included in the description.

The hamster (*Cricetulus griseus*) which has been extensively used in experimental leishmaniasis does not seem prone to develop eye complications. MELENEY (1925) says in regard to the eye: "Parasitized cells are found only in the loose outer layers of the posterior sclera where it joins the orbital connective tissues." Regarding the brain, he writes: "Neither in the brain tissue itself nor in the walls of the intracerebral blood vessels are any parasitized cells found. In the meninges and choroid plexus, however, they are rather numerous, usually in the internal cells of capillaries." ADLER and THEODOR (1934) report the absence of parasitized macrophages in the brains of dogs and different species of hamsters, though in some of the latter parasites were seen in the cells of the choroid plexus.

### SUMMARY

The results of inoculating twenty-five Australian marsupials of the family Phalangeridae, *etc.*, twenty-four specimens of *Trichosurus vulpecula* and one *Pseudocheirus lamiginosus* with *Leishmania donovani* are recorded.

These marsupials were found to be susceptible to kala-azar, twenty-three out of the twenty-five animals were definitely infected.

The experimental disease was established after a single injection of material containing either the flagellate (cultural) or the non-flagellate form of the parasite. For transmission of the non-flagellate form, blood from an infected animal was generally used.

The established disease was of a chronic and progressive nature. Death resulted after periods varying from 3 to 24 months. The clinical manifestations included those of ophthalmic and nervous disease. Parasitic invasion of the eye and of the central nervous system was a common finding. This is in sharp contrast to human kala-azar and the recorded findings in experimental animals.

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## THE OCCURRENCE OF TICK-BORNE TYPHUS IN WEST AFRICA

BY

G M FINDLAY

AND

G T L ARCHER \*

The occurrence of murine typhus throughout West Africa has now been amply demonstrated by the isolation of rickettsiae in guineapigs and by rickettsial agglutination and complement fixation tests (FINDLAY *et al*, 1943, FINDLAY and ELMES, 1947)

In addition, in 1945 there occurred in Northern Nigeria an outbreak of louse-borne typhus. Rickettsiae were isolated in guineapigs from batches of lice in Jos and from the blood of patients in the Bauchi Plateau and from Kaduna. The sera of these patients produced agglutinins and complement fixing antibodies to louse-borne typhus rickettsiae in higher concentrations than to murine typhus rickettsiae (FINDLAY and ELMES, 1947). No complement fixing antibodies were present to tick-borne rickettsiae.

In the present communication a brief description is given of what appear to be cases of tick-borne typhus occurring on the Bauchi Plateau in Nigeria and in Northern Ashanti on the Gold Coast.

No previous description of tick-borne typhus from West Africa has been published, but LONGLEY (1945) has briefly described a fatal disease among dogs in Nigeria possibly due to rickettsiae, and states that he himself suffered from an attack of typhus following the bite of a tick which was believed to have come from a dog. Unfortunately, no further steps were taken to verify the diagnosis of tick-borne typhus in this case. Tick-borne typhus is, of course, well known in South Africa and in Kenya.

### CLINICAL NOTES

The three cases of what are believed to be infections with tick-borne typhus all occurred in the last 3 months of 1943, at the end of the rains.

Two cases occurred in European NCOs attached to a West African battalion stationed in Kintampo in Northern Ashanti, Gold Coast. Kintampo is surrounded by orchard bush.

\* Our thanks are due to the Staff of the Royal Army Medical Corps Emergency Vaccine Laboratory, Everleigh, Wilts, and to Dr J GEAR, South African Institute of Medical Research, Johannesburg, for the supply of rickettsial suspensions and for help in carrying out agglutination and complement fixation tests.



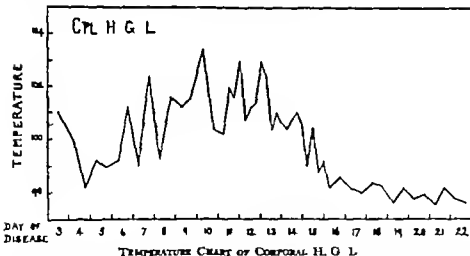
## CASE 1

Cpl. H.G.L., R.A.M.C., age 36 years, Army service 14 years was admitted 21.12.43 complaining of severe frontal headache and sweating. H. had felt off colour for 2 days, but had had no rigors or joint pains. No malarial parasites were seen in the blood. H. was diagnosed as influenza, but on 23.12.43 blotchy pinkish rash appeared first on the front of the body and later on the face. These spots, which were rose-pink, rested on diffuse erythematous base; they faded on pressure. There was no coryza but headache still continued and was worse at night so that the patient could not sleep. By 26.12.43 the patient looked toxic, with a flushed congested face. The urine was normal, but the faeces contained a few macrophages, blood and mucus.

28.12.43 Headache was still the major complaint and sleeplessness. Anorexia was present. The urticula and fauces were reddened and finely granular. Occasional rhonchi were noted in both lungs while the tip of the spleen was just palpable below the left costal margin. The pulmonary second sound was reduplicated. The post-occipital and right epitrochlear lymph nodes were enlarged. The skin showed fine pink rash on the trunk and pink macules sparsely scattered on the trunk. One or two macules were also present on the arms and thighs. Total leucocytes, 11,800 per c.mm. polymorphonuclear leucocytes, 79 per cent, small lymphocytes, 14 per cent. mononuclears, 6 per cent.; eosinophils, 1 per cent.

31.12.43 Headache was still present, but insomnia was less. Tongue white. Chest now clear. The urine contained a slight trace of albumin but on bile. No evidence of any sechar was found.

2.1.44 The temperature was normal for the first time.



6.1.44 The rash had now almost gone except for few macules on the abdomen which faded on pressure leaving faint brown mark. The tongue was now clean and rather raw at the edges.

9.1.44 W.H. good appetite rash now gone no adenitis. The temperature is shown in the Chart.

## CASE 2.

C.Q.M.S. A.B. aged 38. Army service 19 years.

27.12.43. At 22.00 hours complained of nausea and retching.

28.12.43. Felt feverish, muzzy and rather light-headed but continued on duty.

29.12.43. Similar symptoms.

30.12.43. Complained of nausea and severe headache admitted to hospital.

31 12 43 Felt worse, severe postocular and occipital headache, backache, nausea and vomiting Temperature, 101° F, constipated, cough, with whitish yellow sputum, weakness and anorexia

1 1 44 A rigor, temperature, 103° F No malaria parasites seen in blood

2 1 44 A rash noted on the front of the chest and abdomen

3 1 44 The rash is now better defined, especially on the trunk and upper arms, it is of a scarlatiniform type but rose-pink in colour, in addition, there are spots about 4 to 7 mm in diameter and slightly raised, some pink, others a dusky purplish red They are seen on the chest, abdomen and back but more especially on the flanks Only a few are present on the upper arms A few rhonchi are scattered over the chest The epitracheal, posterior cervical and femoral lymph nodes are palpable but not tender

5 1 44 Tip of spleen just palpable, still complains of headache, constipated, tongue heavily furred

6 1 44 Slightly cyanosed and muzzy, frontal headache and insomnia, rash beginning to fade with faint brown marks on pressing the spots

7 1 44 Tongue furred with bluish red, raw edges, slight generalized adenitis

10 1 44 Feels better, temperature now normal, spots very indistinct Total leucocytes, 10,500 per c.mm, polymorphonuclear leucocytes, 47 per cent, small lymphocytes, 41 per cent, mononuclears, 5 per cent, eosinophils, 1 per cent

Convalescence uneventful

### CASE 3

Capt O, age 28 Army service, 3½ years, stationed on the Jos Plateau in Northern Nigeria

22 10 43 Complained of headache and feeling "off colour" The headache continued with considerable severity for 8 days, a rash appeared on the abdomen and chest on the 4th day In appearance, it resembled those described in the other two patients Rhonchi were present in the chest Malaria parasites were not seen in the blood stream The temperature returned to normal on the 14th day

### SEROLOGICAL REACTIONS

The results of serological tests on these three cases are shown in the table on page 818

### EXPERIMENTAL INVESTIGATIONS

Blood from Case 3, removed on the 10th day of illness, was inoculated intraperitoneally into guineapigs which showed a thermal reaction above 104° F on the 8th and 11th days The infection was twice passaged in guineapigs, but further study was impossible owing to the infection of the stock guineapigs with a salmonella. Although no definite scrotal reactions were seen, the tunica vaginalis of male guineapigs was reddened and infected and smears stained by Giemsa's method showed a few rickettsiae in the cytoplasm of endothelial cells

The patient's dog gave a Weil-Felix reaction by the slide test with OX 2 but not with OX 19 or OXK (Agglutination +++ in a dilution of 1 in 2, a trace in a dilution of 1 in 10)

Ticks, *Rhipicephalus sanguineus*, collected both at Bauchi and at Kintampo, gave no reactions when inoculated into guineapigs

### DISCUSSION

The evidence that these cases were due to tick-borne typhus is dependent on the occurrence of a positive Weil-Felix reaction associated with a typhus-like

infection, on the absence of agglutinins and complement fixing antibodies in the sera for murine and epidemic typhus rickettsiae and on the presence of complement fixing antibodies for South African tick typhus.

TABLE  
SEROLOGICAL REACTIONS IN SUSPECTED TICK-BORNE TYPHUS

Number of case.	Weil-Felix reaction.	Rickettsial agglutination.		Epi- demic.	Murine	Tick typhus S.A.
		Epi- demic.	Murine.			
1	1 in 30 1 in 30 0 (8th day)					
	1 in 30 1 in 900 1 in 450 (11th day)					
	1 in 80 1 in 120 1 in 120 (16th day)	1 in 40	1 in 20	1 in 80	1 in 80	1 in 200
	1 in 80 1 in 140 1 in 80 (11th day)	1 in 40	1 in 20	1 in 120	1 in 120	1 in 200
2	1 in 40 1 in 600 1 in 40 (10th day)	1 in 20	1 in 20	1 in 120	1 in 120	1 in 400
	1 in 40 1 in 800 1 in 80 (14th day)					
	1 in 40 1 in 640 1 in 40 (14th day)			1 in 40	1 in 80	1 in 200

1 Cases 1 and 2 the Weil-Felix reactions disappeared in from 10 to 1 weeks after the onset of the illness, but the rickettsial agglutination was obtainable.

Much further investigation is required to determine whether all the forms of tick borne fever south of the Sahara are identical and how far their vectors differ in South, East and West Africa.

There is already evidence that South African tick borne typhus and the *fièvre boutonneuse* of the Mediterranean area are related.

#### CONCLUSIONS

Three cases of typhus are reported in Europeans from West Africa. There is reason to believe that these cases were due to tick-borne rickettsiae which appear to be related antigenically to those responsible for South African tick typhus.

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## HUMAN DICROCOELIASIS IN NIGERIA

BY

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A specimen of human liver sent in connection with the viscerotomy service of the Yellow Fever Research Institute, showed, on section, a small trematode lying in an intrahepatic bile duct. By chance, the section was an almost true median longitudinal section through the worm. The parasite measured 3.8 mm in length, and showed very marked development of the acetabulum, 0.34 mm in diameter, this being larger than the oral sucker, which measured only 0.26 mm in diameter. The operculated eggs were golden-brown in colour, and measured  $42\mu$  in length by  $24\mu$  in width. The only pathological change was a moderate degree of fibrous thickening of the portal tract.

The parasite was identified as a species of *Dicrocoelium*, probably *D. dendriticum* (See plate, page 820).

This appears to be the first record of human dicrocoeliasis from West Africa, though VAN DEN BERGHE and DENECKE (1938) report the parasite as occurring in man and monkeys in the Belgian Congo. MACGREGOR (*in litt*) reports that *Dicrocoelium* sp. occurs as a not infrequent parasite of sheep and oxen in Nigeria, but its exact status is unknown.

I am indebted to the DIRECTOR OF MEDICAL SERVICES, Nigeria, for permission to publish this note.



Ova of *Dicrocoelium*.  
( $\times 430$ .)



Section of liver showing  
trematode, *Dicrocoelium*,  
and fibrosis of the portal  
tract ( $\times 40$ .)

# REFERENCE.

VAN DEN BERGHE L. & DEN CHA, R. 1938) *Ann Soc belge Med trop* 18 509

# A NOTE ON BILHARZIASIS IN WEST AFRICAN TROOPS

BY

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Five hundred and thirty-two soldier recruits, aged between 19 and 25 years, were admitted to a Northern Nigerian hospital during the 19 months between September, 1940, and March, 1942, from a nearby central training unit

Seventy-four had schistosomiasis (18 per cent), fifty-nine being pure *Schistosoma haematobium* infestation, eleven pure *S. mansoni*, and four combined *haematobium* and *mansoni* infestations. One soldier with combined infestation died in hospital, 60 per cent of the infested group had concomitant hook-worm and ascarid infestations, 5 per cent had subtertian rings in the peripheral blood. Haemoglobin levels ranged between 65 and 85 per cent, erythrocyte counts were never below 4,000,000 per cm<sup>3</sup>, eosinophilia ranged from 6 to 19 per cent.

All were treated with anthiomaline and a concluding course of sodium antimony tartrate. Response to anthiomaline alone was invariably slow.

## COMMENTARY

These recruits had previously been medically examined on enlistment in their home districts, where bilharzia infested individuals were either rejected or underwent treatment before acceptance into the central unit. In this way the ordinarily high infestation rate in men from endemic areas such as Fortlami, Zaria, Kano, Yola, Yelwa, Jebba and Sokoto was considerably reduced.

It is probable, therefore, that the 18 per cent figure either represented infestations overlooked on enlistment, or relapses following inadequate treatment. A third possibility, namely re-infestation, or primary infestation after enlistment, seems ruled out by a figure so low as 18 per cent for if, as usually happens, infestation was contracted during manoeuvres, far greater numbers would have been involved. At the same time, through frequent transfer of recruits from this unit, other infested individuals probably escaped detection till much later. At the commencement of the late war it was generally considered that bilharziasis caused little physical impairment among recruits. This proved

incorrect. Infested men soon broke down under prolonged stress, particularly on long marches. Apart from haematuria—infrequent in long infested adults—there was commonly a distinctive triad of symptoms—

- (1) Backache in one or both loins (2) caecal tenderness (3) mucoid diarrhoea.

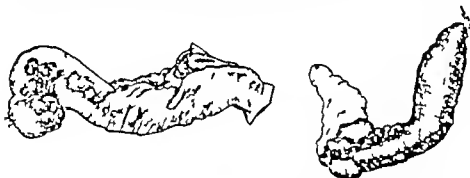
Lumbago-like pains during marches compelled many of them to enter hospital.

In cases where backache predominated, cystoscopy usually revealed vesical bilharzial lesions—bullous-like masses encroaching on one or both ureteral orifices—a condition beautifully illustrated in a case of CHRISTOPHERSON'S.

Caecal tenderness, common in *haematobium* as well as *mansoni* infestations, points to large bowel, and possible appendicular, involvement as well. One soldier in the group under discussion had gross *haematobium* lesions of the appendix.

In such cases the mesh of great omentum may be spotted like a veil with aggregations of innumerable ova.

Numbers of bilharzial appendices have from time to time been removed in London hospitals to the puzzlement of surgeons, until section and microscopic examination have revealed their true pathology.



TWO BILHARZIAL APPENDICES REMOVED AT OPERATION IN AN ENDEMIC AREA OF NORTHERN NIGERIA.

Both show characteristic distortion and surface nodulation with pseudo-tubercles.

In an endemic area of bilharzians in the Sokoto Province of Northern Nigeria, thirty five appendicectomies disclosed a 57 per cent. infestation rate.

Appendicectomy is usually deemed necessary in cases where (1) Caecal tenderness persists after specific drug therapy (2) Radiography shows (a) imperfect filling or delayed emptying (b) an appendix retrocaecally placed.

CHRISTOPHERSON, J. B. & OCHER, WARD, R. (1934) Bilharzia disease in England, *British J Surg* 21 633

## NOTES ON NERVOUS AND MENTAL DISEASES ENCOUNTERED IN NIGERIA

BY

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The object of this paper is to describe the incidence of the nervous and mental disorders found in Nigerian natives, as it has been noticed that there are many differences both in the incidence and manifestations of these diseases, when compared with what is found in practice in the British Isles

There are many difficulties in the diagnosis of nervous diseases in the tropics apart from the difficulty of obtaining reliable pathological and other aids to bedside diagnosis, the African can rarely give an objective account of his symptoms and is usually extremely vague as to their duration, in addition he tends to rationalize his symptoms more than the European and often produces very bizarre explanations, so that the difficulty of taking an accurate clinical history makes the diagnosis tend more to depend on the physical findings on examination than on the history of the disease, this is especially unsatisfactory when dealing with nervous diseases where the diagnosis in many cases depends so much on an accurate history. This difficulty is reflected in the figures given from the annual reports

These notes therefore are merely impressions gained at the bedside in various African hospitals during the course of the last 12 years, and for comparison, figures have been taken from the *Annual Reports of the Medical and Health Services of Nigeria*, 1935 to 1938

Since for statistical purposes the diagnoses have to conform with a limited number of categories based on the *International List of the Causes of Death* (1931), and in many cases these categories are mere dumping grounds of clinically similar syndromes of diverse aetiology—it follows that considerable latitude must be allowed in interpreting many of the diagnoses recorded

\* I have to thank Dr G B WALKER, Director of Medical Services, Nigeria, for permission to publish this paper



## NERVOUS DISEASE

In Table I it is shown that during the four years (1935 to 1938) a total of 3,331 recorded cases of nervous diseases were treated in African Hospitals. They are classified in the table as in the *Annual Reports* in the categories shown with the numbers in each category and as percentages of the total cases.

It appears that certain diseases such as disseminated sclerosis, tabes dorsalis, and chorea are comparatively rare, although the first is now one of the

TABLE I.

Code number	Disease.	Number of cases.	Per cent.
16	Acute poliomyelitis	13	0.6
17	Encephalitis lethargica	14	0.4
18	Cerebrospinal fever	641	19.2
24	Tuberculosis of the central nervous system	15	0.4
78	Encephalitis, cerebral abscess (not including encephalitis lethargica)	45	1.4
79	Meningitis (not including tuberculous or cerebrospinal meningitis)	181	4.8
80	Tabes dorsalis	80	1.8
81	Other diseases of the spinal cord	73	2.2
82	(1) Cerebral haemorrhage apoplexy	120	3.6
	(2) Cerebral embolism	21	0.6
	(3) Cerebral thrombosis	23	1.6
	(4) Hemiplegia and other paralysis of un stated origin	873	26.2
85	Epilepsy	632	19.3
87	(a) Chorea	13	0.4
	(b) Neuritis, neuralgia	293	11.8
	(c) Disseminated sclerosis	18	0.5
	(d) Other diseases of the central nervous system	148	4.4
Total number of recorded diseases		3 331	100.0

commonest chronic organic nervous diseases in the British Isles (WALSH, 1941). The low incidence of chorea is interesting, though judging by the number of cases of mitral stenosis encountered, acute rheumatism cannot be so rare in the tropics as is usually believed.

Although acute anterior poliomyelitis would appear to be rare from the figures—judging by the number of cases of residual paralysis met with, it appears to be commoner than it is in Europe—most of these cases in the hospitals having probably been classified in the category of "Hemiplegia and other paralysis of un stated origin." The incidence may be higher owing to the

comparatively low standard of sanitation, recent research having shown that the virus may be excreted in the faeces and transmitted in sewage (KLING *et al*, 1942)

Another disease which appears to be rare is cerebral tumour, though the incidence of malignant tumours generally is lower than in Europe. Symptoms of increased intracranial tension usually denote internal hydrocephalus following suppurative meningitis, usually cerebrospinal fever, of which annual epidemics regularly occur especially in the Northern Provinces in the dry season, during the winter and spring months, and which constitute in the table no less than 19.2 per cent of the total nervous diseases, and taking meningitis as a whole comprise almost a quarter of the total admissions during the 4 years.

Polyneuritis associated with paraesthesiae, muscular wasting and tenderness and loss of tendon reflexes is common. This is probably in many cases due to malnutrition and avitaminosis B<sub>1</sub>, though toxins from helminthic infestation may play some part, and acute infective polyneuritis is found. A typical polyneuritis also sometimes occurs in leprosy, the incidence of which is very high, and it must be differentiated from the local nerve involvement of neural leprosy.

Primary optic atrophy is common and in many cases may be due to syphilis, though again malnutrition is probably responsible for a large number of cases, and others are due directly or indirectly to trypanosomiasis.

Acute encephalitis occurs rarely as a complication of the acute exanthemata, especially smallpox, it also occurs in malaria, especially in infants, and in a recent epidemic of louse-borne typhus it was one of the most pronounced features of the disease. Cerebral abscess is common largely as a result of a neglected middle ear disease. Occasional cases of post-encephalitic parkinsonism are seen, though a very similar syndrome is common as a result of cerebral syphilis.

Cerebrospinal syphilis is very common, especially in the areas where the primary disease is found. It is almost invariably of the meningo-vascular type which is in extreme contrast to the rarity of the so-called parenchymatous type of infection, tabes dorsalis and general paresis (G.P.I.). Possibly this is associated with the theory that late syphilis attacks those parts of the organism which are subjected to excessive strain, which would account for the relative immunity of the African's central nervous system to late syphilitic manifestations compared with his cardio-vascular system, as syphilitic aortitis and aneurysm are relatively common. Possibly also repeated attacks of malaria exert a prophylactic effect against the subsequent development of general paresis in syphilitics. Whatever the reason, symptoms of G.P.I. in Nigerian natives, when found, are more likely to be due to trypanosomiasis than to syphilis.

Motor neurone disease occurs and is usually of the chronic bulbar palsy type, though classical cases of progressive muscular atrophy are occasionally

seen. Confusion in diagnosis, however is common unless these cases are followed up, as meningo-vascular syphilis produces a very similar syndrome, and the response to specific therapy or a long follow up period is the only criterion by which the underlying pathology can be determined.

Transverse myelitis is most commonly due to syphilis, producing various degrees of paraplegia, though minor degrees of paraplegia are also very common following spinal deformity due to spinal caries or trauma.

Idiopathic epilepsy appears to be as common as it is in the United Kingdom, though it appears in Nigeria to be more commonly associated with some degree of dementia this is possibly due to the fear with which the epileptic is usually regarded and the consequent sense of inadequacy which is thereby engendered in him. In Table I it will be noticed that epilepsy accounted for over 20 per cent. of the total admissions, though a large proportion of these were probably cases of fits with an underlying organic basis such as hypertensive attacks, trauma or trypanosomiasis.

### MENTAL DISEASES.

In the diagnosis of mental disorders the same difficulties of obtaining an adequate history are found as in the case of nervous diseases, and these again are reflected in the figures in Table II which have been taken from the same

TABLE II.

Code number	Disease.	Number of cases.	Per cent
83	General paralysis of the insane (G.P.I.)	27	1.3
84	( ) Dementias praecox	26	1.2
	(b) Paranoia	31	1.7
	(c) Other forms of insanity	1,814	89.8
	(d) Asementia	40	1.1
	( ) Hysteria	162	5.4
87	Psychasthenia, neurasthenia	153	8.1
Total number of recorded diseases		1,874	100.0

*Animal Reports as Table I* In addition, in the tropics mental disease appears in many varied and different forms and, owing to the high incidence of concurrent tropical diseases such as malaria, trypanosomiasis, helminthiasis, malnutrition and hypovitaminosis, the clinical picture, especially of the organic psychoses, is very much more confused than it is in Europe and in treatment it is first necessary to deal with every underlying concurrent physical disorder before a final prognosis can be considered in the majority of the conditions found, or even in many cases a correct diagnosis made thus, Table II

it will be seen that over 80 per cent of all the cases of mental disorders have been classified in the category of "Other causes of insanity"

A high proportion of the mental disorders encountered is found to consist of the "functional" disorders, or psychoneuroses, these forming 13.5 per cent of the total admissions, and this proportion is almost certainly too low as the incidence of these is very high as is to be expected in a primitive people steeped in magic. The incidence of the different types of functional disorder in the population is interesting among the most primitive, uneducated by Western standards, hysteria and obsessional states are the usual manifestations, whereas in the more highly educated African, who has been subjected to the possibilities of severe conflict produced by the impinging of Western ideas on the magical beliefs of his forefathers, anxiety states are relatively common though unknown in the more primitive "bushman". Suggestibility is very high in these latter and the response to treatment on the whole is very much more satisfactory than in the semi-educated African with an anxiety state. In some cases dramatic improvement has been obtained by suggestion under narcosis with intravenous barbiturates.

The major psychoses, examples of all types of which are to be found in the asylums, are common, though those with an organic basis are commoner than in the British Isles, with the exception of G.P.I., which is relatively uncommon, as previously described. The incidence of schizophrenia and the major affective psychoses does not appear to be so common.

Of the organic psychoses, delirium and acute confusional states are extremely common as a result of malaria or lobar pneumonia, stupor and coma in the later stages are the usual manifestations of trypanosomiasis.

Senile and hypertensive conditions are less common, first because the expectation of life in the African is considerably less and relatively few live beyond the age of 60 years, and secondly because arterial sclerosis and degeneration are less common.

Postencephalitic states are encountered from time to time associated with Parkinsonism, thyroid insufficiency, in certain parts of the country, results in a certain proportion of cretins and cases of myxoedema, and varying degrees of idiocy and amentia are common in areas where trypanosomiasis occurs.

Episodic attacks of mania occur occasionally in women during pregnancy and lactation, though involutional crises associated with puberty and the menopause are rare. Typical pellagra is very rare, though malnutrition is almost universal and in bad cases is sometimes associated with stupor or confusion.

Manic-depressive insanity occurs, and it is interesting that the manic phase appears to be more often seen than the depressive, in many cases it is associated with religious or magical delusions, though as all magic is delusional in logical Western eyes, the diagnosis of delusions in Africans is fraught with difficulty, and there is no doubt that many cases appearing at first sight to belong to the category of manic-depressive insanity, are in reality to be classed among the functional hysterical or obsessional states.

The same difficulty is found in the diagnosis of schizophrenia and the systematized delusional states, though typical dementia præcox is seen and is the only type in this group which is relatively easy to diagnose, though the apparent stupor and lack of interest may in reality be due to trypanosomiasis, the possibility of which in every psychosis must always be present in the mind of the clinician.

These impressions are given very diffidently as the difficulties of exact diagnosis in bush stations in Africa are considerable, as has been described. It is hoped that the opinions expressed may stimulate interest in a difficult and somewhat neglected subject of study in the tropics.

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WALKER, F. M. B. (1941) *Diseases of the Nervous System*. Edinburgh E. & S. Livingstone 162.

## HAEMOGLOBIN AND PLASMA-PROTEIN LEVELS IN WEST AFRICA

BY

J D STEPHEN, M B, CH B, B SC, CAPTAIN, R A M C \*

A comparative study was made of the haemoglobin and total plasma protein levels in West Africans. The prevalence of dietary deficiencies and anaemias, nutritional and otherwise, in West Africa is well known. The primary purpose of the investigation was to compare the haemoglobin and plasma-protein levels in troops of the Royal West African Frontier Force with those of the civilian population, in order to determine whether army rations had any effect in increasing haemoglobin values.

Seven groups were studied, each group consisting of fifty persons.

GROUP 1—African Army recruits, ages 20 to 30 years, service, 6 weeks. These men were mostly from the Northern Territories of the Gold Coast.

GROUP 2—African soldiers, ages 20 to 30 years, with 6 to 8 months' service, recruited from all parts of the Gold Coast.

GROUP 3—African soldiers, ages 23 to 38 years, with 2 or more years' service. All these men had been in India and had been on a European-scale ration, many had taken suppressive mepacrine. Recruited from all parts of the Gold Coast.

GROUP 4—African villagers, males, ages 20 to 30 years. From the coastal district of the Gold Coast.

GROUP 5—African children, male and female, ages 3 to 14 years. From the coastal district of the Gold Coast.

GROUP 6—African women during pregnancy, ages 18 to 39 years. From the coastal district of the Gold Coast.

GROUP 7—European soldiers, ages 21 to 44 years. Stationed in Accra, Gold Coast, for from 3 to 18 months.

### TECHNIQUE

Five ml of venous blood was withdrawn into a mixture of 6 mg of ammonium oxalate and 4 mg of potassium oxalate. The specific gravity of the whole blood and of the plasma was then determined by the copper sulphate method (PHILLIPS *et al*, 1943). The haemoglobin and plasma-protein levels were then read from a simple line chart.

\* I wish to thank Brigadier G M FINDLAY, CBE, for advice and help in this investigation.

## RESULTS

The results of the survey are shown in Tables I and II. The highest mean level of haemoglobin was found in Group 7 consisting of European soldiers: the results compare favourably with levels observed in surveys in Great Britain. All these soldiers were on suppressive mepacrine and few if any had had malaria. The high level of haemoglobin in the African soldiers of Group 3 is probably due to the European-scale diet enjoyed by these soldiers.

TABLE I.

HEMOGLOBIN IN AFRICANS AND EUROPEANS IN THE GOLD COAST WEST AFRICA.

Group.	1	2	3	4	5	6	7
Range (in grams per 100 mL)	10.4-16.4	11.2-16.4	13.4-17.2	11.4-17.4	11.1-14.9	7.1-13.7	12.4-17.2
Mean (in grams per 100 mL)	13.2	14.2	16.1	14.2	12.2	11.4	15.4
Standard deviation	1.15	1.22	1.37	1.34	1.15	1.73	1.63

TABLE II.

TOTAL PLASMA-PROTEIN IN AFRICANS AND EUROPEANS IN THE GOLD COAST WEST AFRICA.

Group.	1	2	3	4	5	6	7
Range (in grams per 100 mL)	6.8-9.3	6.1-8	6.1-8.3	6.5-9.6	5.8-8.3	5.8-7.6	5.4-8.1
Mean (in grams per 100 mL)	7.6	7.4	7.2	7.3	7.1	6.6	6.8
Standard deviation	0.69	0.66	0.67	0.6	0.71	0.41	0.61

for a considerable period and to the fact that while in India they had had little or no malaria. The haemoglobin level of the male African villagers (Group 4) is approximately 1 per cent. higher than that of the recruits of Group 1 but this may not be a true estimate, as the recruits were all men from the Northern Territories of the Gold Coast, while the villagers were drawn from the coastal belt where the food supply is more plentiful than in the Northern Territories. A truer comparison is provided by soldiers of Group 2, where there was no segregation into districts. The low level in pregnant women is not surprising

in view of the frequency of macrocytic anaemia. The level in children is no lower than is to be expected, in view of the ubiquitous malarial attacks to which they are subject.

The plasma-protein levels are more difficult to interpret. While the values for total proteins are on a level with or even higher than those recorded in Europeans, it is by no means certain that the albumin-globulin ratio is the same as that of Europeans. The work of MOHUN (1946) has shown that there may be a distortion of the albumin-globulin ratio in the West African and that quite often the albumin level is lower than the globulin. It was not possible in this investigation to do differential albumin and globulin estimations. Table II gives the level of plasma-proteins in the seven groups examined, there are no significant variations.

#### CONCLUSION

A study has been made of haemoglobin and plasma-protein levels in West Africa. It appears that the haemoglobin level in natives is generally lower than that of the European, and that the highest African level is to be found in those troops who had been given a European-scale ration and in many cases suppressive mepacrine. It is thus possible to effect a rise in the African's haemoglobin level so that it equals that of the European. No significant difference between African and European plasma-protein levels was noted.

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APPOINTMENT OF EDITOR  
OF THE  
TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

It is with much regret that the Council of the Society announces that Miss MILDRED WENTON who has so long been connected—27 years—with the Royal Society of Tropical Medicine and Hygiene, and with its TRANSACTIONS has decided to resign from her post as Assistant Editor on the completion of the current volume. A tribute was paid to Miss WENTON in Vol. 40 No. 6 (July 1947), and it would be redundant to enlarge on that. The Society's debt to Miss WENTON cannot be over estimated. To her more than to any other person can be attributed the high standard which the publication of this journal has attained.

On learning of Miss WENTON's decision, the Council of the Society decided to appoint a Scientific Editor and is happy to announce that Lieut.-General Sir WILLIAM P. MACARTHUR, K.C.B. D.S.O. O.B.E. M.D. F.R.C.P. D.T.M. & H., has agreed to accept this post, beginning with the first number of Vol. 42. Sir WILLIAM MACARTHUR is well known to Fellows of the Society and needs no introduction. He will be assisted in his duties by Mr. S. H. PILTON who will act as Publishing Consultant.

## ANNOUNCEMENTS.

### NEXT MEETING OF THE SOCIETY

The 41st Annual General Meeting of the Society will be held at Manson House, 26, Portland Place, London, at 7 30 p m, on Thursday, 17th June, 1948, when the Chalmers Medals for 1941, 1943 and 1945 will be presented

An Ordinary Meeting will follow at 8 15 p m Professor MAHOMED ERFAN, F R C P (LOND), Egypt, will read a paper on Pulmonary bilharziasis

### MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are temporarily in the British Isles Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26, Portland Place, London, W 1, can usually be forwarded to the home address

To ensure the accuracy of this list, Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

ABBOTT, H, Sudan  
AL-ABED, H E, Iraq  
AWOLIYI, S O, Nigeria  
BRANCH, A G W, Nigeria  
CAFFREY, P J, Nigeria  
CARMAN, J A, Kenya  
CHARTRES, J C, Nigeria  
CLARK, MILDRED, Natal  
CUMMINS, Lt -Col P H, India  
DAVIDSON, Lt -Col T J, India  
DAVIES, C W, Kenya  
DICK, G W R, Uganda  
DIXON, P, Belgian Congo  
DUGGAN, A, Nigeria  
FARMAN-FARMAIAN, S, Persia  
FOY, HENRY, Greece  
GRAHAM-CUMMING, G, Hongkong  
HARDING, R D, Nigeria  
HOLMES, R E, Belgian Congo  
HUGHES, M H, Gold Coast  
HUNTER, W, Nigeria  
IP, YEE, Hongkong  
JONES, S A, Palestine  
KELSEY, H A, Nigeria  
KENT, Lt -Col P W, India  
KHAYYAT, S, Irak  
KONDI, A, Greece  
LINDSAY, Lt -Col D K LL, India

MCIPHERSON, D R, Malaya  
MILLS, A R, Entrea  
MORTON, T A, Gold Coast  
MTAWALI, C V, Tanganyika  
NELSON, J W, Northern Rhodesia  
PAL, RAJINDAR, India  
PHILLIPS, C M, Northern Rhodesia  
QUANTRILL, D W, Nigeria  
RITCHIE, G L, Tanganyika  
RUSSELL, S FARRANT, Pakistan  
SAUNDERS, G F T, Gold Coast  
SCRIMGEOUR, H, Singapore  
SEIN, Lt -Col MIN-, Burma  
SIMPSON, T, Nigeria  
SMITH, Lt -Col M C L, India  
THOMSON, FLORENCE A, Malaya  
To, SHIU-YUEN, Hongkong  
UTTLEY, K H, Hongkong  
WATERMAN, JAMES, Trinidad  
WATT, Lt -Col GEORGE, Gold Coast  
WHITE, T H, Tanganyika  
WIGGAN, W C, Nyasaland  
WILKINS, E G, India  
WILSON, C J, Kenya  
WILSON, W A, Uganda  
WING, W M, U S A  
WRIGHT, F J, Kenya

## NEW FELLOWS

At the meeting of the Society held at Mansion House on 15th April, 1948 the following twenty-one candidates were elected Fellows of the Society —

ABBOTT TERENCE R., M.B. B.S. (SYD.) D.P.H. (LOND) Seychelles.  
 AGRAWAL, JAGDESH P. M.B. B.S. (PATNA) India.  
 BLECHKA, W. M.D. (POLAND) England.  
 CASTILLO C. M.D. D.T.M. & H., South America.  
 COMBESONCO, L. M. M.B., Ch.B. (EDIN.) British West Indies.  
 CURRIE GORDON M.B. Ch.B. (GLAS.) Nysaland.  
 FARQUHARSON A. D. J. M.B. Ch.B., L.R.C.P. England.  
 FOX, IRVING M.A. (GEORGE WASHINGTON UNIV.), PH.D. (IOWA), Puerto Rico.  
 HEDDINGTON FRANK H. B.Sc. (MIDDLEBURY) M.D. (NEW YORK) China.  
 HOLLIS, ARTHUR M., B.A. (ONTARIO). Completing work for M.Sc. in Parasitology (Tulane) Canada.  
 JOPLING W. H., M.B. Ch.B. (ENG) L.R.C.P. (LOND) D.T.M. & H., Southern Rhodesia.  
 KARMARER, B. C. M.B. (CALCUTTA) Pakistan.  
 LI, ROBERT MAN-KIM, M.B., B.S. (HONGKONG), Hongkong.  
 McWHIRTER, D. L. B.Sc. (EDIN) M.B. Ch.B. Shanghai.  
 MONTGOMERY, A. M., M.B., Ch.B. (EDIN.) South Africa.  
 NESTLE, A., M.D. (AMMAN) D.C.H. (DUBLIN) Iraq.  
 POT, ARNOLD W. M.D. (UTRECHT) Netherland West Indies.  
 SODMAN WM. A. M.D. B.S., F.A.C.P. (MICHIGAN) U.S.A.  
 SIU KA HEE, M.B., B.S. (HONGKONG) Hongkong.  
 TENG, PETER M. M.D. (CANADA) China.  
 TORKILDSEN F. BUCH 10 (OSLO) England.

## ADMISSION TO FELLOWSHIP OF THE SOCIETY

All registered medical and veterinary practitioners and others interested in scientific pursuits relating to tropical medicine, whose qualifications are deemed satisfactory by the Council, are eligible for election as Fellows of the Society.

Anyone desiring to become a candidate for Fellowship of the Society should use the special form of application at the end of this Journal.

The annual subscription payable by Fellows is one and half guineas (£1 11 6d.) which becomes due in advance on the 1st of April of each year.

The TRANSACTIONS and the current YEAR BOOK of the Society are posted regularly to every Fellow whose subscription is not in arrears.

Further information may be obtained from the Hon. Secretaries, Mansion House 26 Portland Place, London, W 1 or from the Local Secretary of the district.

## NOTICE TO FELLOWS

A copy of each number of the TRANSACTIONS is posted to every Fellow whose subscription is not in arrears. Fellows are particularly requested to notify the Secretaries of any change in the address to which their TRANSACTIONS are to be posted.

When copies of the TRANSACTIONS are returned by the Post Office marked "Gone Away", "No Service", or "Insufficient Address", no more copies will be posted to that address but they will be retained at Mansion House until further instructions are received.

## SCIENTIFIC INFORMATION CONFERENCE

The Royal Society has arranged, from 21st June to 2nd July, 1948, a Scientific Information Conference in London

The Conference will be attended by representatives of countries providing information services in English, namely, the countries of the British Commonwealth and the United States of America

The Sections and Editors-in-chief are as follows —

Section 1 Publication and distribution of papers reporting original work  
Professor J D BERNAL, F R S

Section 2 Abstracting Services Sir DAVID CHADWICK

Section 3 Indexing and other library services Dr J E HOLMSTROM

Section 4 Reviews, Annual Reports, etc Professor H MUNRO FOX, F R S

Application for tickets of admission should be made in writing before 1st June, to the Assistant Secretary, The Royal Society, Burlington House, London, W 1, from whom further particulars of the Conference may also be obtained

## PRIZE TO BE AWARDED IN 1948

### THE CONSULTANTS PRIZE

The Consultants to the War Office and the Armies in the Field in the late war, have presented a sum of money to the R A M C in order to found a Consultants Prize, to be competed for at intervals of 1 to 3 years

This prize will be awarded for the first time in 1948 and will be to the value of 25 guineas. The prize is open to serving officers of the Royal Army Medical Corps, holding a regular or a short-service commission

The first prize will be awarded for an essay of not more than 10,000 words on a professional subject, based on the author's own experiences between 1939 and 1946. It is hoped that these essays will ensure that valuable war experience, which would otherwise be lost, will be recorded for future guidance and possibly for publication

Entries should be sent in through the usual channels, so as to reach the Hon Secretary, R A M C Prize Funds Committee, R A M College, Millbank, London, S W 1 not later than 1st August, 1948

## LIBRARY NOTICES

From the Society's file of the *International Journal of Leprosy*, Volumes 8 & 9 (1941-1942) are missing, and are not obtainable from the publishers, the original stock having been destroyed in Manila

Of the *Annales de la Société Belge de Médecine tropicale*, No 1 of Volume 19 (1939) is missing

Of the *Chinese Medical Journal*, Volumes 62 & 63 (1944, 1945) are lacking, no numbers having been received

Of the *Journal of Tropical Medicine & Hygiene*, No 4 of Volume 49 (1947) is missing

Gifts of any of the missing volumes or numbers will be gratefully received at Manson House

## NEW BOOK RECEIVED

*The Lepromin Test* BELRA Medical Series, No 1

## WAR DAMAGED LIBRARIES POST WAR RESTORATION

Fellows will be rendering a service to the Society if they return to Manson House any numbers of the TRANSACTIONS which they do not wish to keep—particularly any numbers of Volumes 40.

The Council wishes to thank those Fellows who have already responded by returning copies of the TRANSACTIONS.

## PHOTOSTAT SERVICE

To help Fellows of the Society who cannot readily obtain access to medical periodicals and journals arrangements have been made to supply Photostat copies of papers required.

By courtesy of the Dean and Council of the London School of Hygiene and Tropical Medicine the Library of the School has been placed at our disposal for this purpose. Copies will be made by Photostat Lantern.

Fellows will be asked to pay only the actual cost of preparing the Photostat copy. The minimum charge is 1s. per page copied (size 6½ x 11½) but a price list will be sent on request.

Application should be made to the Secretary Royal Society of Tropical Medicine and Hygiene, Manson House 26 Portland Place W 1 giving the name of the journal, volume and page number of the article required. Complete papers or specified portions of a paper can be supplied, but in the latter case lucid instructions must be given.

## STUDIES ON LIVER DAMAGE IN ACUTE MALARIA

Vol 41 (4) 555 January 1949

Dr J. KLEBERG has recently written (too late for alteration in Vol. 4) to say that he wishes the order of the names of the authors of this paper to be reversed, and the paper be taken as by Dr D. BURNHAM and Dr J. KLEBERG, (instead of by Dr KLEBERG and Dr BURNHAM).

# Royal Society of Tropical Medicine and Hygiene.

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Qualifications, etc.,  
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Name in Full  
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\* When both addresses are given the address to which it is desired that communications be sent should be underlined.

PROPOSER

SECONDER

Signature  
of Fellow

Signature  
of Fellow

1. "Extracts from Laws of the Society No 8—" Either the proposer or the seconder must have personal knowledge of the candidate and vouch for him as in every respect suitable for election as a Fellow of the Society " No 24—" Every Fellow shall pay an Annual Subscription of One-and-a-half Guineas (£1 1s. 6d) " No 25—" The name of a newly elected Fellow shall not be placed on the Register of Fellows nor shall he be entitled to any of the privileges of Fellowship until after his first annual subscription (£1 1s 6d) or composition fee (£23 12s 6d) shall have been paid "

### DECLARATION BY CANDIDATE

I hereby undertake, if elected, to observe and obey the Laws and Regulations of the Royal Society of Tropical Medicine and Hygiene, and to endeavour to promote its honour and interests

Signature of Candidate

19

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## PUBLICATIONS OF THE SOCIETY

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*Back Numbers* Owing to the greatly increased demand for complete sets of the TRANSACTIONS, the stock of certain numbers had been exhausted These have now been reprinted, and any number can be supplied

### YEAR BOOK, 1948

List of Fellows (with Addresses) Alphabetically and Geographically arranged The Society's Annual Reports, Laws, and other matter  
Price Five Shillings, *post free*

### MONOGRAPH

Monograph I (September, 1936) "Boomerang Leg and Yaws in Australian Aborigines," by C J HACKETT 66 pages, 17 pages plates, stiff board cover, linen back  
Price 5s, *post free*

### "MANSON CENTENARY"

Proceedings of meeting at Manson House on 12th December, 1944, with coloured portrait, price Two Shillings and Sixpence, *post free*

### "THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE"

An illustrated pamphlet by "Onlooker," describing the work and functions of the Society and the amenities of Manson House  
Supplied free on application to the Secretary

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Orders for the TRANSACTIONS and other publications of the Society should be sent direct to the Secretary, Royal Society of Tropical Medicine, Manson House, 26, Portland Place, London, W 1 *Telegrams* Anopheles, London *Telephone* Langham 2127 Cheques, Postal Orders, etc., should be made payable to the Royal Society of Tropical Medicine and Hygiene

The TRANSACTIONS may also be ordered through Messrs H K Lewis and Co., Ltd, 136, Gower Street, London, W C 1, or any other bookseller

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### ADVERTISEMENTS

Approved Announcements are accepted by the Council for insertion in the TRANSACTIONS, due to be published in alternate months as follows —

No 1	July 25th	No 4	January 25th
No 2	September 25th	No 5	March 25th
No 3	November 25th	No 6	May 25th

Advertisement type area 5 inches wide, 7½ inches deep (approx)

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All communications regarding advertisements should be addressed to the "Advertising Manager"



## EDITORIAL NOTICES

Papers submitted for publication in these TRANSACTIONS should be sent to the Hon Secretaries, Mansel House 26, Portland Place London W.1

The submission of matter for publication will be understood to imply that it is offered to this journal alone.

If accepted for publication, the copyright of papers becomes the property of the Society but they may be re-published by permission of the Council, provided due acknowledgment be made of their having appeared in the TRANSACTIONS.

Papers should, if possible, be typewritten they should be concisely written with subject matter logically arranged and sub-divided with references and abbreviations the form described below and with indications of the position, in the text, of illustrations, tables, maps, etc.

They should be as brief as consistent with clarity and in many cases the value of paper enhanced by short summary at the end.

Temperature charts, graphs and drawings should be if possible in Indian ink on Bristol board; with detail and essential lettering large enough to be clearly legible after reduction if necessary (Write in pencil if lettering on drawing is to be set up and printed.)

Illustrations—if the number sent on is considered excessive, the author may be informed and given the opportunity of contributing to the cost.

Coloured plates are made only at the author's expense.

## REFERENCES

In the text, the date of publication, in brackets, should follow the name of the author quoted thus—

T. MASON (1878) is due this epoch-making discovery

At the end of the paper list of References should be arranged in alphabetical order of authors surnames, and details given in the following order—(1) Surname of author; (2) Initials of author; (3) Year of publication, in brackets; (4) Title of article, avoiding arbitrary capitals. (The title of the article is sometimes omitted but each list of references should in this respect be consistent throughout—giving all titles, or omitting all). (5) Title of journal; (6) Volume number; (7) Page number e.g.—MASON, P. (1878) On the development of *Plasmodium falciparum* and on the sporozoites considered as oocysts. *J. Lond. Soc. Zool.*, 14, 304.

In the case of reference to book (1), (2) and (3) as above (4) Title of book; (5) Edition and/or volume, if more than one; (6) Page number; (7) Title of publication; (8) Publisher's name, e.g.—MASON, P. (1895). *Tropical Diseases*, 1st Ed. 447 London Cassell & Co., Ltd.

Reference to an ANNUAL REPORT SWAZILAND (1927). *Annual Medical & Sanitary Report 1926* p. 18

Not The year of publication is not usually the year covered by the Report.

## ABBREVIATIONS

The abbreviations used are those shown in the World List of Scientific Periodicals 1931 which conforms to the rules of the International Code of Abbreviations for Titles of Periodicals Paris, 1920. In general nouns have capital, adjectives small, initial letters, articles, conjunctions and prepositions are omitted the place of emphasis is added only when uncertainty might arise e.g.

Amer. J. Hyg.	C. R. Acad. Sci., Paris.	J. Pharmaceutical
Ann. trop. Med. Parasit.	C. R. Acad. Sci. Johannesburg.	Arch. Tübinger Geront.
Arch. Schiffs. Tropenhyg.	Dtsch. med. Woch.	Trans. R. Soc. trop. Med. Hyg.
Bull. Soc. Path. exot.	Indian med. Gaz.	Z. Hyg. Infektkr.

The following contractions are so use whether the number to be expressed is 1 or more, e.g. 1 cc., 16g., 43 kg.)—

centigramme, cg.	kilogramme, kg.	millilitre, ml.
centimetre, cm.	kilometre, km.	millimetre, mm.
cubic centimetre, c.c.	micron, $\mu$ .	ounce, oz.
cubic millimetre, c.mm.	milligramme, mg.	pound, lb.

In order to avoid dangerous error in dosage "grain" and "grams" are printed in full.

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